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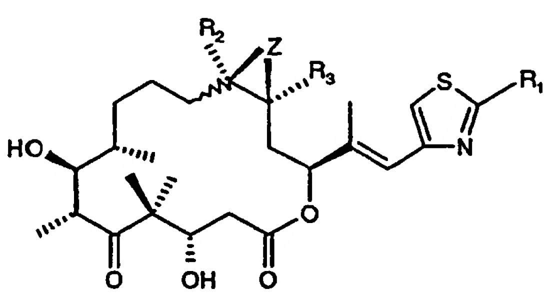
(54) Title: 13-ALKYL EPOTHILONE DERIVATIVES



(IA)

(57) Abstract: The invention relates to novel 13-lower alkyl epothilones of formula (IA); wherein R_1 is methyl, hydroxymethyl, halomethyl, methylthio or methoxy; R_2 is hydrogen or methyl, R_3 is lower alkyl, and Z is O or a bond; intermediates and a process for the preparation of such 13-lower alkyl epothilones and epothilones in general; a pharmaceutical composition comprising such 13-lower alkyl epothilones and the use of such compounds for the treatment of tumor diseases; a method of treatment of warm-blooded animals; catalytic antibodies having aldolase activity and a process for enantioselectively resolving a racemic mixture of aldol synthons by means of antibody catalyzed retro-aldol reaction.

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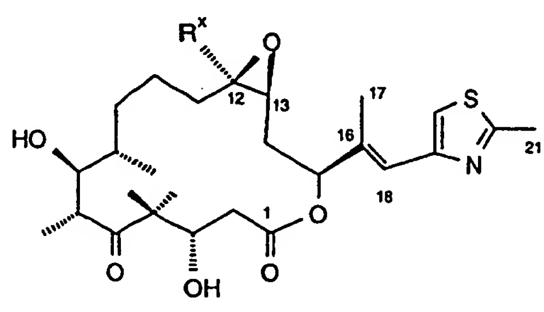
13-Alkyl Epothilone Derivatives

The invention relates to 13-lower alkyl epothilones; intermediates and a process for the preparation of epothilones and 13-lower alkyl epothilones; a pharmaceutical composition comprising 13-lower alkyl epothilones; the use of such compounds for the treatment of tumor diseases; a method of treatment of warm-blooded animals; catalytic antibodies having aldolase activity; a process for enantioselectively resolving a racemic mixture of aldol synthons by means of antibody catalyzed retro-aldol reaction.

A number of chemical reactions have been catalyzed by monoclonal antibody catalysts. Most of those transformations were catalyzed with very high enantio- and regioselectivity. Several reactions were rerouted from their normal pathway and disfavored reactions were catalyzed. Recently, two aldolase monoclonal antibodies 38C2 and 33F12 were generated against a β-diketone hapten, 6-(4-glutaramidophenyl)-hexane-2,4-dione, using reactive immunization (J. Wagner, R.A. Lerner, C.F. Barbas III, Science 1995, 270, 1797). In reactive immunization highly reactive chemicals are used as immunogens so that a chemical reaction occurs in the binding site of an antibody during its induction. An analogue of this chemical reaction later becomes part of a catalytic event.

Epothilones A and B represent a new class of microtubul e-stabilising cytotoxic agents (see Gerth, K. et al., J. Antibiot. 1996, 49, 560-3) of the formula:



wherein R^X means hydrogen (epothilone A) or methyl (epothilone B).

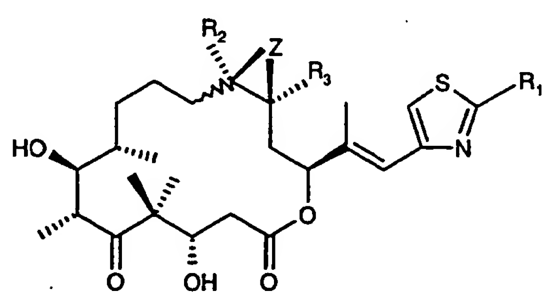
These compounds have advantages over Taxol®, a branded product already introduced for the treatment of tumours, that has th same mechanism of action but has however a series

of disadvantages, such as very poor water solubility, making the preparation of pharma - ceutical formulations very difficult (at present, such formulations are normally characterised by the toxic side effects of the carrier materials), and inefficacy on a series of tumours. The advantages are as follows:

a) They have better water-solubility and are thus more readily accessible for formulations.
b) It has been reported that, in cell culture experiments, they are also active against the proliferation of cells, which, owing to the overexpression of the P-glycoprotein efflux pump are "multidrug resistant", i. e., show resistance to treatment with other chemotherapy agents including Taxol® (see Bolag, D. M., et al., "Epothilones, a new class of microtubule-stabilizing agents with a Taxol-like mechanism of action", Cancer Research 1995, 55, 2325-33). And
c) it could be shown that they are still very effective *in vitro* against a Taxol®-resistant ovarian carcinoma cell line with modified β-tubulin (see Kowalski, R. J., et al., J. Biol. Chem. 1997, 272(4), 2534-2541).

Pharmaceutical application of the epothilones, for example for tumour treatment, is possible in an analogous manner to that described for Taxol, see, for example, US 5.641.803; US 5.496.804; US 5.565.478. One disadvantage of the epothilones is the relatively low therapeutic index, i.e. the dosage range between the necessary dose and the maximum tolerable dose is very small.

Surprisingly it has now been found that compounds of formula I



(I)

in which compounds

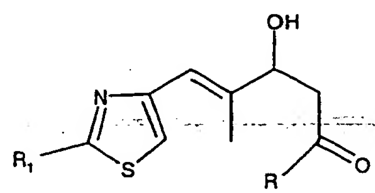
R_1 is methyl, hydroxymethyl, halomethyl, methylthio or methoxy;

R_2 is hydrogen or methyl,

R_3 is hydrogen or lower alkyl, and

Z is O or a bond;

can be obtained from chemical intermediates II obtained by a process of enantioselectively resolving a racemic mixture of an aldol synthon (±)-II



((±)-II)

using catalytic antibodies.

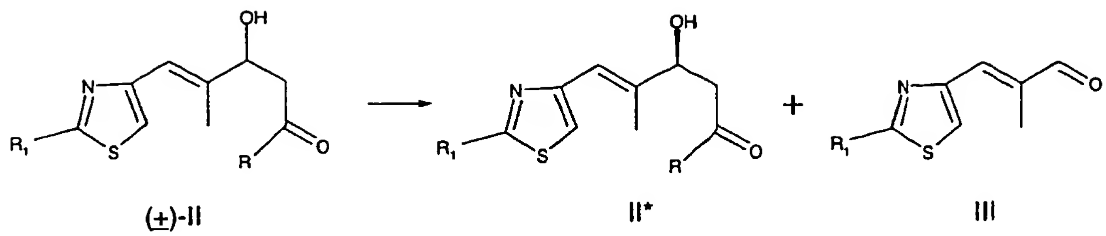
Therefore, the invention relates to a process for enantioselectively resolving a racemic mixture of an aldol synthon, the racemic mixture including a first and a second enantiomer of the aldol synthon, said process comprising the following steps:

Step A: Catalyzing a retro-aldol reaction for enantioselectively converting the first enantiomer of the aldol synthon to form an aldehyde product while leaving the second enantiomer of the aldol synthon unmodified, said catalyst employing a catalytic antibody; and

Step B: Separating the aldehyde product from the unmodified second aldol synthon.

In this process the racemic mixture need not to be a 50/50 mixture of the two enantiomers.

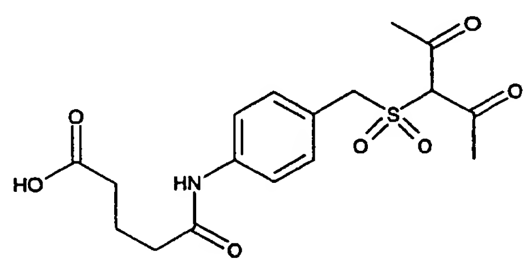
Especially, the invention relates to a process wherein the racemic mixture of the aldol synthon of formula (±)-II is resolved to provide an unmodified enantiomer of formula II* and an aldehyde of formula III



Preferably, in this process a catalytic antibody selected from the group consisting of 84G3, 85H6 and 93F3 is employed.

Preferably, in formula (±)-II R_1 represents methyl, hydroxymethyl, halomethyl, methylthio or methoxy, and R represents C_{1-5} alkyl, n-but-1-en-4-yl or halomethyl. More preferably, R_1 represents methyl, hydroxymethyl, fluoromethyl, methylthio or methoxy, and R represents methyl, ethyl, n-propyl, n-butyl, n-pentyl, n-but-1-en-4-yl or fluoromethyl. Most preferably, R_1 represents methyl, hydroxymethyl, methylthio or methoxy, and R represents methyl, ethyl, n-propyl, n-butyl, n-pentyl or n-but-1-en-4-yl.

Catalytic antibodies 84G3, 85H6 and 93F3 were generated through immunization of mice against the hapten of formula IV



(IV).

coupled to the carrier protein keyhole limpet hemocyanine (KLH) according to the procedures described by J. Wagner et al, Science 1995, 270, 1798 and G. Kohler et al, Nature 1975, 256, 495. The antibodies were purified according to the methods described by V.E. Gouvernour et al, Science 1993, 262, 204, and deposited with the American Type Culture Collection (ATCC), 10801 University Blvd, Manassas, VA 20110-2209, USA on October 07, 1999 by The Scripps Research Institute under the deposit designations PTA-823, PTA-824 and PTA-825, respectively. 84G3 and 93F3 operate with a catalytic

proficiency of $(k_{cat}/K_m)/k_{un} > 10^{13}$ M^{-1}. These new catalysts present significant advantages with regard to the synthesis of epothilone and their analogs.

It is disclosed herein that catalytic antibodies 84G3, 85H6 and 93F3 have antipodal reactivity with regard to catalytic antibody 38C2. These three antibodies are especially effective with regard to the catalytic resolution of compounds (±)-II. E.g., the antibodies 84G3 is used in such resolution in a multi-gram scale in a quantity of between 0.00001 and 0.5 mol%.especially 0.0004, 0.003 or 0.005 mol%.

Deposits for hybridoma 84G3, 85H6 and 93F3 were made in compliance with the Budapest Treaty requirements that the duration of the deposits should be for 30 years from the date of deposit at the depository or for the enforceable life of a patent that matures from this application, whichever is longer. The assignee of the present application has agreed that if the hybridoma deposit should die or be lost or destroyed when cultivated under suitable conditions, it will be promptly replaced on notification with a viable specimen of the same hybridoma.

Furthermore, the present invention relates to a process for the preparation of a compound of formula I, in which

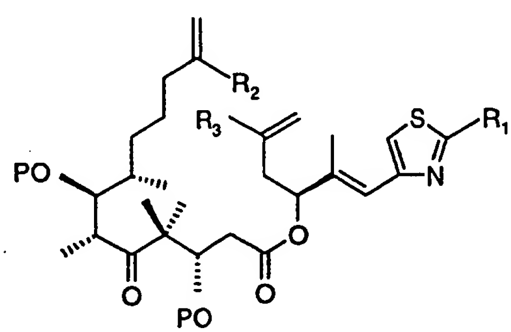
R_1 is methyl, hydroxymethyl, halomethyl, methylthio or methoxy;

R_2 is hydrogen, methyl,

R_3 is hydrogen or lower alkyl, and

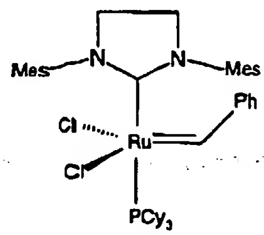
Z is O or a bond;

wherein a compound of formula V,



(V)

in which P is *tert*-butyl-dimethylsilyl or another suitable protecting group for a hydroxy group, R_1 is methyl, hydroxymethyl which is protected by tert-butyl-dimethylsilyl or another suitable hydroxy protecting group, halomethyl, methylthio or methoxy, and the other radicals have the meaning as given above for formula I, is transformed into a compound of formula I by an olefine metathesis reaction in the presence of the catalyst of formula VI



(VI)

in which compound of formula V Mes represents mesityl and Ph phenyl, followed by the detaching of the protecting group by a suitable reagent, and in which process directly before or after detaching the protecting groups present a compound of formula I wherein Z represents a bond can optionally be transformed by epoxidation in analogy to the procedure described in Example 26 of WO99/43653 into a compound of formula I wherein Z represents O, and, after carrying out the above process, if necessary for the preparation of a salt, converting a resulting free compound of the formula I into a salt or, if necessary for preparation of a free compound, converting a resulting salt of a compound of the formula I into the free compound.

It is a further objective of the invention to provide epothilone derivatives, which, through their advantageous biological and pharmacological properties, enable the armamentarium for the control of, in particular, proliferative diseases such as tumours to be expanded. Also, compounds have to be found, which have an improved therapeutic index compared with epothilones A and B.

Surprisingly, 13-lower alkyl epothilone are pharmacologically highly effective for the indications mentioned herein. Hence, the invention relates also to 13-lower alkyl epothilone of formula IA wherein

R_1 is methyl, hydroxymethyl, halomethyl, methylthio or methoxy;

R_2 is hydrogen, methyl,

R_3 is lower alkyl, and
Z is O or a bond;

If Z represents a bond, a double bond is established between the both carbon atoms wearing the radical Z.

The compounds of formula IA and their pharmaceutically acceptable salts have advantageous pharmaceutical properties. For example, they are active against multidrug-resistant cell lines and tumours and/or they have an improved therapeutic index over natural epothilones.

The general terms used hereinbefore and hereinafter preferably have within the context of this disclosure the following meanings, unless otherwise indicated:

The prefix "lower" denotes a radical having up to and including a maximum of 7, especially up to and including a maximum of 5 carbon atoms, the radicals in question being either branched with single or multiple branching or unbranched.

Where the plural form is used for compounds, salts, and the like, this is taken to mean also a single compound, salt, or the like ("a" as an indefinite article or as a numeral meaning "one").

Asymmetric carbon atoms that are optionally present in the substituents may exist in the (R), (S) or (R,S) configuration, preferably in the (R) or (S) configuration. Substituents on a double bond or on a ring may be present in cis- (=Z-) or trans- (=E-) form. The present compounds may thus exist as mixtures of isomers or as pure isomers, preferably as pure diastereoisomers.

Lower alkyl has mono- or multiple-branching or, preferably, is unbranched or and is in particular methyl, ethyl, propyl, especially n-propyl, or butyl, especially n-butyl. Very preferably lower alkyl is methyl or ethyl.

Halogen is especially fluorine, chlorine, bromine, or iodine, in particular fluorin or chlorine.

Halomethyl is fluoromethyl or chloromethyl, in particular fluoromethyl.

Preferably, in the compound of formula IA
R_1 is methyl, hydroxymethyl, halomethyl, methylthio or methoxy;
R_2 is hydrogen or methyl,
R_3 is lower alkyl, and
Z is O or a bond.

More preferably, in the compound of formula IA
R_1 is methylthio;
R_2 is hydrogen;
R_3 is lower alkyl, especially methyl or ethyl and
Z is O or a bond, prefeably a bond.

Furthermore, the present invention provides compounds of formula II*, which are useful intermediates for the preparation of epothilone derivatives of formula I.

In such compound of formula II*, preferably R_1 represents methyl, hydroxymethyl, halomethyl, lower alkoxy lower alkylenoxy methyl, preferably methoxy methylenoxy methyl, methylthio or methoxy, and R represents lower alkyl, n-but-1-en-4-yl or halomethyl. Very preferably, R_1 represents methylthio, and R represents methyl or ethyl.

Salts of compounds of formula I with a salt-forming group may be prepared in a manner known *per se*. Acid addition salts of compounds of formula I may thus be obtained e.g. by treatment with an acid or with a suitable anion exchange reagent.

Salts can usually be converted to free compounds, e.g. by treating with suitable basic agents, for example with alkali metal carbonates, -hydrogencarbonates, or -hydroxides, typically potassium carbonate or sodium hydroxide.

The macrocyclisation of a diene of formula V wherein P is tert-butyl-dimethylsilyl or another suitable hydroxy protecting group, R_1 is methyl, hydroxymethyl which is protected by tert-butyl-dimethylsilyl or another suitable hydroxy protecting group, halomethyl, methylthio or

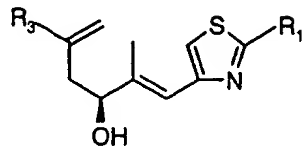
methoxy; R_2 is hydrogen or methyl, and R_3 is hydrogen or lower alkyl, to a compound of formula I, wherein the radicals P, R_1, R_2 and R_3 have the same meaning as in the compound of formula V and Z is a bond, takes place, preferably under the following conditions: The compound V is solved in a suitable, dry solvent or mixture of solvents with a boiling point between 30 and 60 °C, e.g., dichloro methane. The catalyst of formula VI is added, preferably, in an amount of 5 mol % to 30 mol %, very preferably between 15 and 25 mol %, e.g., 20 mol %, and the cyclisation is carried out, e.g., at the reflux temperature of the solvent for a duration of 12 to 120 h, preferably 20 to 96 h. The reaction can be controlled by NMR analysis of the reaction mixture.

Starting materials

The starting materials are known, may be produced by known processes or are commercially available, or they may be produced as described in the following:

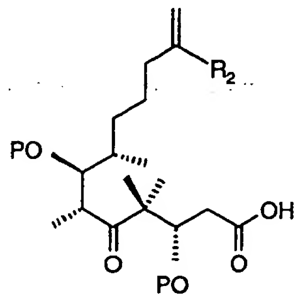
In the following preparation processes for intermediates, functional groups which are to be in protected form can be protected if necessary at suitable stages, whereby selective protection or deprotection is also possible. The protecting groups and the methods of introducing and/or removing them correspond to those named above under process a), especially those named in the above-mentioned standard reference works or, in particular, in the examples. As a rule, protecting groups are not mentioned in the following; the following examples show where the usage of the protecting groups is appropriate or necessary and can therefore be regarded as a preferred instruction as to when protecting groups should be used and if compounds should be produced with other radicals. In the following, protecting groups are not mentioned at all the points where they are appropriately used. The person skilled in the art is clear as to where this usage ought to or must occur.

A compound of formula V wherein P is tert-butyl-dimethylsilyl or another suitable hydroxy protecting group, R_1 is methyl, hydroxymethyl which is protected by tert-butyl-dimethylsilyl or another suitable hydroxy protecting group, halomethyl, methylthio or methoxy; R_2 is hydrogen or methyl, and R_3 is hydrogen or lower alkyl, can be obtained by the esterification of an alcohol of formula VII



(VII)

wherein the radicals R_1 and R_3 have the same meaning as in the compound of formula V, with an acid of formula VIII

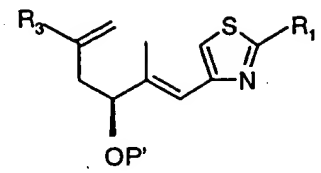


(VIII)

wherein P is tert-butyl-dimethylsilyl or another suitable hydroxy protecting group and R_2 has the same meaning as in the compound of formula V. The esterification can be carried out under conditions known *per se*, especially in the presence of a dehydrating agent like DCC (dicyclohexylcarbodiimide). For example, the acid can be solved in a suitable, inert and dry solvent, e.g. tetrahydrofuran or, preferably, dichloromethane, together with the alcohol at a temperature between - 10 °C and room temperature, e.g., 0 °C. Optionally, EDC and DMAP (4-(dimethylamino)-pyridine) can be added and the mixture is stirred for 12 to 24 h, e.g. 16 h, at the same temperature.

The preparation of an acid of formula VIII wherein R_2 is hydrogen or methyl and P is *tert*-butyl dimethylsilyl, is, e.g., disclosed in WO 98/08849, especially in the Example on page 30, the subject-matter of which is hereby incorporated into the present application by reference to this publication.

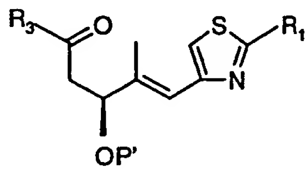
An alcohol of formula VII wherein the radicals R_1 and R_3 have the same meaning as in the compound of formula V, can be obtained by the treatment a compound of formula IX



(IX)

wherein the radicals R_1 and R_3 have the same meaning as in the compound of formula VII and P' is a protecting group, with a reagent suitable to detach the protecting group. E.g., if P' is *tert*-butyl dimethylsilyl, treatment of IX with TBAF in a suitable solvent, like tetrahydrofuran, at a temperature between 0°C and room temperature, provides the unprotected alcohol VII. If R_1 comprises a hydroxy group protected by the same protecting group P', the treatment mentioned before also detaches the protecting group in R_1. The protecting group *tert*-butyl dimethylsilyl can be reintroduced by reacting VII with TBSCl (*tert*-butyl dimethylsilyl chloride) in the presence of diisoproylethylamine in a suitable solvent, like, e.g., dichloromethane, at a temperature between 0 °C and room temperature for 6 to 12 h, e.g., 8 h.

A compound of formula IX is obtainable by a Wittig reaction of a compound of formula X

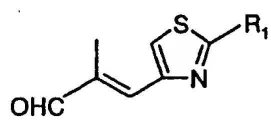


(X)

wherein the radicals R_1, R_3 and P' have the same meaning as in the compound of formula IX using, e.g., the Wittig reagent methyl-triphenylphosphonium iodide. The Wittig reaction is known as such. In the present case, the reaction can be carried out in dry tetrahydrofuran or another suitable solvent at temperatures between -10 °C and + 30 °C, preferably between 10 °C and room temperature. After adding buthyl lithium to the reagent methyl-triphenylphosphonium iodide at 0 °C, the solution is stirred for about 30 minutes at room temperature. Then the compound of formula X is added and the solution is stirred for further 15 to 90 minutes at the same temperature.

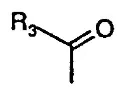
A compound of formula X can be prepared by reacting a compound of formula II wherein the radicals R_1 and R_3 have the same meaning as in the compound of formula X, with a reagent suitable to introduce a protecting group P', e.g. with TBSCl, in a suitable solvent, especially dimethylformamide, preferably in the presence of imidazole, at room temperature

Compounds of formula (±)-II can be prepared by the reaction of a compound of formula XI



(XI)

wherein the radical R_1 has the same meaning as in the compound of formula (±)-II, with a compound of formula XII



(XII)

wherein the radical R_3 has the same meaning as in the compound of formula (±)-II, in which reaction the compound of formula XII is first solved in dry tetrahydrofuran and a solution of LDA is added at -78 °C. After stirring for between 1 and 4 h, the solution is cooled further to a temperature of about -100 °C and the compound of formula XI is added at the same temperature. The reaction mixture is stirred for further 30 to 120 minutes and then quenched by the addition of a suitable acidic reagent, e.g. an aqueous solution of NH_4Cl.

General process conditions

Stereoisomeric mixtures, e.g. mixtures of diastereoisomers, can be separated into their corresponding isomers in a manner known *per se* by means of suitable separation methods. Diastereoisomeric mixtures may thus be separated into their individual diastereoisomers by means of fractionated crystallization, chromatography, solvent distribution, and similar procedures, preferably according to the purification procedures described in the Examples. This separation may take place either at the stage of one of the starting compounds.

Enantiomers may be separated through the for mation of diastereoisomeric salts, for example by salt formation with an enantiomer-pure chiral acid, or by means of chromatography, for example by HPLC, using chromatographic substrates with chiral ligands. (Enantiomer separation is normally effected at the interme diate stage).

All process steps described here can be carried out under known reaction conditions, pre - ferably under those specifically mentioned, in the absence of or usually in the presence of solvents or diluents, preferably those that are inert to the reagents used and able to dissol - ve them, in the absence or presence of catalysts, condensing agents or neutralisi ng agents, for example ion exchangers, typically cation exchangers, for example in the H^+ form, depending on the type of reaction and/or reactants at reduced, normal, or elevated temperature, for example in the range from -100°C to about 190°C, preferably from about - 80°C to about 150°C, for example at -80 to 60°C, at room temperature, at - 20 to 40°C or at the boiling point of the solvent used, under atmospheric pressure or in a closed vessel, if required under pressure, and/or in an inert, for example an argon or nitrogen, atmosphere.

Salts may be present in all starting compounds and intermediates, if these contain salt-forming groups. Salts may also be present during the reaction of such compounds, provided that the reaction is not thereby disturbed.

At all reaction stages, isomeric mixtures that occur can be separated into their individual isomers, e.g. diastereoisomers or enantiomers, or into any mixtures of isomers, e.g. racemates or diastereoisomeric mixtures, for example analogously to methods described under "Additional process steps".

In certain cases, typically in dehydrogenation or aldol reactions, it is possible to achieve stereoselective reactions, allowing for example easier recovery of individual isomers.

The solvents from which those can be selected which are suitable for the reaction in question include for example water, esters, such as lower alkyl-lower alkanoate, e.g ethyl acetate, ethers, such as aliphatic ethers, e.g. diethylether, or cyclic ethers, e.g. tetra-hydrofuran, liquid aromatic hydrocarbons, such as benzene or toluene, alcohols, such as methanol, ethanol or 1- or 2-propanol, nitriles, such as acetonitrile, halogenated hydrocarbons, such as methylene chloride, acid amides, such as dimethylformamide,

bases, such as heterocyclic nitrogen bases, e.g. pyridine, carboxylic acids, such as lower alkanecarboxylic acids, e.g. acetic acid, carboxylic acid anhydrides, such as lower alkane acid anhydrides, e.g. acetic anhydride, cyclic, linear, or branched hydrocarbons, such as cyclohexane, hexane, or isopentane, or mixtures of these solvents, e.g. aqueous solutions, unless otherwise stated in the description of the process. Such solvent mixtures may also be used in working up, for example by chromatography or partitioning.

The invention relates also to those embodiments of the process in which one starts from a compound obtainable at any stage as an intermediate and carries out the missing steps, or breaks off the process at any stage, or forms a starting material under the reaction condi - tions, or uses said starting material in the form of a reactive derivative or salt, or produces a compound obtainable by means of the process according to the invention under the pro - cess conditions therein, and further processes the said compound *in situ*. In the preferred embodiment, one starts from those starting materials which lead to the compounds descri - bed hereinabove as preferred, particularly as especially preferred, primarily preferred, and/ or preferred above all.

In the preferred embodiment, compounds of formula I are prepared analogously to the processes and process steps defined in the examples.

The compounds of formula I, including their salts, are also obtainable in the form of hydrates, or their crystals may include for example the solvent used for crystallisation (present as solvates).

The invention relates also to a compound of formula I obtained by the process of preparation disclosed herein.

Salts are primarily the pharmaceutically acceptable salts of compounds of formula I.

Such salts are formed, for example, as acid addition salts, preferably with organic or inor - ganic acids, from compounds of formula I with a basic nitrogen atom, especially the pharmaceutically acceptable salts. Suitable inorganic acids are, for example, hydrohalic acids, such as hydrochloric acid, sulphuric acid, or phosphoric acid. Suitable organic acids are, for example, carboxylic, phosphonic, sulphonic or sulphamic acids, for example acetic

acid, propionic acid, octanoic acid, decanoic acid, dodecanoic acid, glycolic acid, lactic acid, 2-hydroxybutyric acid, gluconic acid, glucosemonocarboxylic acid, fumaric acid, succinic acid, adipic acid, pimelic acid, suberic acid, azelaic acid, malic acid, tartaric acid, citric acid, glucaric acid, galactaric acid, amino acids, such as glutamic acid, aspartic acid, N-methylglycine, acetylaminoacetic acid, N-acetylasparagine or N-acetylcysteine, pyruvic acid, acetoacetic acid, phosphoserine, 2- or 3-glycerophosphoric acid, maleic acid, hydroxymaleic acid, methylmaleic acid, cyclohexanecarboxylic acid, benzoic acid, salicylic acid, 1- or 3-hydroxynaphthyl-2-carboxylic acid, 3,4,5-trimethoxybenzoic acid, 2-phenoxybenzoic acid, 2-acetoxybenzoic acid, 4-aminosalicylic acid, phthalic acid, phenylacetic acid, glucuronic acid, galacturonic acid, methane- or ethane-sulphonic acid, 2-hydroxyethanesulfonic acid, ethane-1,2-disulphonic acid, benzene sulphonic acid, 2-naphthalenesulphonic acid, 1,5-naphthalene-disulphonic acid, N-cyclohexylsulphamic acid, N-methyl-, N-ethyl- or N-propyl-sulphamic acid, or other organic protonic acids, such as ascorbic acid.

For isolation or purification purposes it is also possible to use pharmaceutically unacceptable salts, for example picrates or perchlorates. Only the pharmaceutically acceptable salts or free compounds (if the occasion arises, in the form of pharmaceutical preparations) attain therapeutic use, and these are therefore preferred.

In view of the close relationship between the novel compounds in free form and in the form of their salts, including those salts that can be used as intermediates, for example in the purification or identification of the novel compounds, hereinbefore and hereinafter any reference to the free compounds is to be understood as referring also to the corresponding salts, as appropriate and expedient.

The compounds of formula IA have valuable pharmacological properties, as described hereinbefore and hereinafter.

The efficacy of the compounds of formula IA as enhancers of micro tubule polymerisation may be proved as follows:

Stock solutions of the test compounds (10 mM) are prepared in DMSO and stored at -20°C. Microtubule protein is xtracted from pigs' brain by two cycles of temperature-dep ndent depolymerisation/polymerisation, as known (see Weingarten et al., Biochemistry 1974; 13,

5529-37). Working stock solutions of micro tubule protein (i.e. tubulin plus microtubule-associated proteins) are stored at -70°C. The degree of test-compound-induced poly-merisation of microtubule protein is determined basically as already known (see Lin et al., Cancer Chem. Pharm. 1996, 38, 136-140). Drug or vehicle (DMSO, final concentration 5%) are diluted in 1x MEM buffer (100 mM MES, 1 mM EGTA, 1 mM $MgCl_2$, pH 6.7) and placed in an eppendorf tube on ice. Following additon of microtubule protein (final concentration 1 mg/ml), the polymerization reaction is started by transferring the incubation mixtures to a room-temperature water bath for 5 min. Then, the reaction mixtures are placed in an Eppendorf microcentrifuge and incubated for a further 15 minutes at room temperature. The samples are then centrifuged for 15 minutes at 14,000 rpm at room temperature, in order to separate polymerised from unpolymerised micro tubule protein. As an indirect measure of tubulin polymerisation, the protein concentration of the su pernatant (which contains the remaining non-polymerised, soluble micro tubule protein) is determined by the Lowry method (DC Assay Kit, Bio-Rad Laboratories, Hercules, CA, USA), and the optical density (OD) of the colour reaction is measured at 750 nm using a spectrometer (Spectra Max 340, Molecular Devices, Sunnyvale, CA, USA). The difference in OD's between samples treated with a test compound and vehicle-treated controls is compared with that obtained with incubations containing 25 µM epothilon B (positive control). The degree of polymerisation induced by a test compound is expressed relatively to the positive control (100%). In this test compounds of formula I exhibit a degree of polymerisation of 2 to 78 %, in particular 39% to 78% for compounds of formula IA wherein R_1 is methylthio.

The efficacy against tumour cells may be demonstrated in the following way:

Stock solutions of the test compounds (10 mM) are prepared in DMSO and stored at -20°C. Human KB-31 and (multidrug-resistant, P-gp170 expressing) KB-8511 epidermoid carcino-ma cells originate from Dr. M. Baker, Roswell Park Memorial Institute (Buffalo, NY, USA) (description: see also Akiyama et al., Somat. Cell. Mol. Genetics 1985, 11, 117-126 and Fojo A., et al., Cancer Res. 1985, 45, 3002-3007 - KB-31 and KB-8511 are both derivati ves of the KB cell line (ATCC) and are human epidermoid carcinoma cells. The cells are cultured as previously described (I. Utz, S. Hofer, U. Regenass, W. Hilbe, J. Thaler, H. Grunicke, and J. Hofmann, Int. J. Cancer 1984, 57, 104). Anti-proliferative assays ar performed as previously described (T. Meyer, et al., Int. J. Cancer 1989, 43, 851). Briefly,

cells are seeded at 1.5 x 10^3 cells/well into 96-well microtiter plates and incubated overnight. Compounds are added in serial dilutions on day 1. The plates are than incubated for an additional 4 days, after which the cells are fixed with 3.3 % v/v glutaraldehyde, washed with water and stained with 0.05% w/v methylene blue. After washing, the dye is eluted with 3 % HCl and the optical density measured at 665 nm with a SpectraMax 340 (Molecular Devices, Sunnyvale, CA, USA). IC50 values are determined by mathematical curve-fitting using the Softmax program currently version 2.6 (Molecular Devices, Sunnyvale, CA, USA) using the formula (OD treated - OD start) / (OD control - OD start) x 100. The IC50 is defined as the drug concentration which leads to 50 % of cells per well compared to control cultures at the end of the incubation period. Compounds of formula IA show for the KB-31 cell line an IC50 in the range of 0.05 and 250 nM, preferably between 1 and 50 nM.

Tests on other tumour cell lines, e.g., A549 (lung; ATCC CCL 185), NCIH460 (lung), HCT-15 (colon; ATCC CCL 225), HCT-116 (colon), MCF-7 (breast; ATCC HTB 22), or Du-145 (prostate; ATCC No. HTB 81) can be carried out in a comparable manner.

The *in vivo* efficacy may be demonstrated as follows: The models used are xeno -transplants of tumours, such as KB-31 or KB-8511 epidermoid tumours, in mice. The anti-tumour efficacy of the test compounds may be measured in female BLB/c nu/nu mice for example against the corresponding subcutaneously transplanted cell line. To this end, tumour fragments of about 25 mg are implanted into the left side of each of the mice (for example 6 animals per dose). The test compound is administered for example on day 11 after transplantation in different dosages (for example 0.1; 0.5; 1; 5 and 10 mg/kg), if desired repeating the administration, if required several times, after between two days and two weeks. The volumes of the tumours are determined for example after about 2 to 4 weeks (e.g. two weeks after the start of treatment). The tumour volumes are calculated by measuring the tumour diameter along two vertically arranged axes and according to published methods (see Evans et al., Brit. J. Cancer 1982, 45, 466-8). The anti-tumour efficacy is determined as the mean increase in tumour volume of the treated animals divided by the mean increase in tumour volume of the untreated animals (controls) and, after multiplication by 100, is expressed as T/C%. Tumour regression (given in %) is calculated as the smallest mean tumour volume (Vt) in r lation to the mean tumour volume at the start of treatment (Vo) ac -

cording to the formula

$$\% \text{ regression} = [1-(Vt/Vo)] \times 100.$$

In this case also, other cell lines can be used, for example those named above in the demonstration of efficacy against tumour cells.

Owing to these properties, the compounds of formula IA are suitable for the treatment of proliferative diseases, especially tumour diseases, including metastases; for example solid tumours such as lung tumours, breast tumours, colorectal tumours, prostate tumours, melanomas, brain tumours, pancreas tumours, neck tumours, bladder tumours, neuroblastomas, throat tumours, but also proliferative diseases of blood cells, such as leukaemia; also for the treatment of other diseases which respond to treatment with micro tubule depolymerisation inhibitors, such as psoriasis. In one preferred embodiment of the invention, the compounds of formula IA are used for the treatment of prostate tumours.

Especially preferred are the compounds named in the examples, or salts thereof (especially pharmaceutically acceptable salts), provided that a salt-forming group is present.

Pharmaceutical preparations, methods, and uses

A compound of formula IA can be administered alone or in combination with one or more other therapeutic agents, possible combination therapy taking the form of fixed combinations or the administration of a compound of the invention and one or more other therapeutic agents being staggered or given independently of one another, or the combined administration of fixed combinations and one or more other therapeutic agents. A compound of formula IA can besides or in addition be administered for tumour therapy in combination with chemotherapy, radiotherapy, immunotherapy, surgical intervention, or a combination of these. Long-term therapy is equally possible as is adjuvant therapy in the context of other treatment strategies, as described above. Other possible treatments are therapy to maintain the patient's status after tumour regression, or even chemopreventive therapy, for example in patients at risk.

Therapeutic agents for possible combination are especially one or more antiproliferative, cytostatic or cytotoxic compounds, for example one or more chemotherapeutic agent (s) selected from the group comprising the classical chemotherapeutic agents, an inhibitor of polyamine biosynthesis, an inhibitor of protein kinase, especially of serine/threo nine protein kinase, such as protein kinase C, or of tyrosine protein kinase, such as epidermal growth factor receptor protein tyrosine kinase, a cytokine, a negative growth regulator, such as TGF-ß or IFN-ß, an aromatase inhibitor, and a classical cytostatic.

The present invention relates also to pharmaceutical compositions that contain a compound of formula IA as active ingredient and that can be used especially in the treatment of the diseases mentioned above. Compositions for enteral administration, such as nasal, buccal, rectal or, especially, oral administration, and for parenteral administration, such as intravenous, intramuscular or subcutaneous administration, to warm-blooded animals, especially humans, are especially preferred. The compositions contain the active ingredient alone or, preferably, together with a pharmaceutically acceptable carrier. The dosage of the active ingredient depends upon the disease to be treated and upon the species, its age, weight, and individual condition, the individual pharmacokinetic data, and the mode of administration.

The invention relates also to pharmaceutical compositions for use in a method for the pro - phylactic or especially therapeutic treatment of warm-blooded animals, including human, especially suffering from a tumour disease, in particular breast cancer or prostate cancer, to a process for the preparation thereof (especially in the form of compositions for the treatment of tumours) and to a method of treating the above-mentioned diseases, primarily neoplastic diseases, especially those mentioned above.

In one preferred embodiment of the invention the tumour disease that is treated is prostate cancer or breast cancer.

The invention relates also to the use of compounds of formula IA for the preparation of pharmaceutical preparations which contain compounds of formula IA as active component.

Furthermore, the invention relates to a compound of formula IA for use in a process for the diagnostic or therapeutic treatment of humans and to the use of a compound of formula IA for the treatment of a tumour disease.

Preference is given to a pharmaceutical composition that is suitable for administration to a warm-blooded animal, especially a human or commercially useful mammal, suffering from a disease that is responsive to the enhancement of micro tubule polymerisation, for example psoriasis or especially a neoplastic disease, comprising a correspondingly effective amount of a compound of formula IA, or a pharmaceutically acceptable salt thereof when salt-forming groups are present, together with at least one pharmaceutically acceptable carrier.

A pharmaceutical composition for the prophylactic or especially therapeutic treatment of neoplastic and other proliferative diseases of a warm-blooded animal, especially a human or a commercially useful mammal requiring such treatment, especially suffering from such a disease, comprising a new compound of formula IA, or a pharmaceutically acceptable salt thereof, as active ingredient in a quantity that is prophylactically or especially therapeutically active against said diseases, is likewise preferred.

Pharmaceutical preparations contain from about 0.000001 % to 95 % of the active ingredient, whereby single-dose forms of administration preferably have from approximately 0.00001 % to 90 % and multiple-dose forms of administration preferably have from approximately 0.0001 to 0.5 % in the case of preparations for parenteral administration or 1 % to 20 % active ingredient in the case of preparations for enteral administration. Unit dosage forms are, for example, coated and uncoated tablets, ampoules, vials, suppositories or capsules. Further dosage forms are, for example, ointments, creams, pastes, foams, tinctures, lipsticks, drops, sprays, dispersions, etc. Dosage unit forms, such as coated tablets, tablets or capsules, contain about 0.01 g to about 2 g, preferably about 0.02 g to about 1.0 g, of the active ingredient, in particular 0.02 to 0.6 g.

The pharmaceutical preparations of the present invention are prepared in a manner known *per se*, for example by means of conventional mixing, granulating, coating, dissolving or lyophilising processes.

Preference is given to the use of solutions of the active ingredient, and also suspensions or dispersions, which, for example in the case of lyophilised preparations which contain the active ingredient on its own or together with a carrier, for example mannitol, can be made up before use. The pharmaceutical preparations may be sterilised and/or may contain excipients, for example preservatives, stabilisers, wetting agents and/or emulsifiers, solubilisers, salts for regulating the osmotic pressure and/or buffers and are prepared in a manner known *per se*.

Suspensions in oil contain as the oil component the vegetable, synthetic, or semi-synthetic oils customary for injection purposes. In respect of such, special mention may be made of liquid fatty acid esters that contain as the acid component a long-chained fatty acid having from 8 to 22, carbon atoms, for example lauric acid, tridecylic acid, myristic acid, pentadecylic acid, palmitic acid, margaric acid, stearic acid, arachidic acid, behenic acid or corresponding unsaturated acids, for example oleic acid, elaidic acid, erucic acid, brassidic acid or linoleic acid, if desired with the addition of antioxidants, for example vitamin E, β-carotene or 3,5-di-tert-butyl-4-hydroxytoluene. The alcohol component of these fatty acid esters has a maximum of 6 carbon atoms and is a mono- or polyhydric, for example a mono-, di- or trihydric, alcohol, or the isomers thereof, but especially glycol and glycerol. As fatty acid esters, therefore, the following are mentioned: ethyl oleate, isopropyl myristate, isopropyl palmitate, "Labrafil M 2375" (polyoxyethylene glycerol trioleate from Gattefossé, Paris), "Labrafil M 1944 CS" (unsaturated polyglycolised glycerides prepared by alcoholysis of apricot seed oil and consisting of glycerides and polyethylene glycol ester; Gattefossé, France), "Labrasol" (saturated polyglycolised glycerides prepared by alcoholysis of TCM and consisting of glycerides and polyethylene glycol ester; Gattefossé, France), and/or "Miglyol 812" (triglyceride of saturated fatty acids of chain length C_8 to C_{12} from Hüls AG, Germany), but especially vegetable oils such as olive oil, cottonseed oil, almond oil, castor oil, sesame oil, soybean oil and more especially groundnut oil.

The manufacture of injectable preparations is usually carried out under sterile conditions, as is the filling, for example, into ampoules or vials, and the sealing of the containers.

Pharmaceutical compositions for oral administration can be obtained, for example, by combining th active ingredient with one or more solid carriers, if need be granulating a resulting

mixture, and processing the mixture or granules, if desired, to form tablets or tablet cores, if need be by the inclusion of additional excipients.

Suitable carriers are especially fillers, such as sugars, cellulose preparations, and/or calcium phosphates, for example tricalcium phosphate or calcium hydrogen phosphate, and also binders, such as starches, for example corn, wheat, rice or potato starch, and/or polyvinylpyrrolidone, and/or, if desired, disintegrators, such as the above-mentioned starches, also carboxymethyl starch, crosslinked polyvinylpyrrolidone, alginic acid or a salt thereof, such as sodium alginate. Additional excipients are especially flow conditioners and lubricants, for example silicic acid, talc, stearic acid or salts thereof, and/or polyethylene glycol, or derivatives thereof.

Tablet cores may be provided with suitable, if need be enteric, coatings, using *inter alia* concentrated sugar solutions which may comprise gum arabic, talc, polyvinylpyrrolidone, polyethylene glycol and/or titanium dioxide, or coating solutions in suitable organic solvents or solvent mixtures, or, for the preparation of enteric coatings, solutions of suitable cellulose preparations, such as acetylcellulose phthalate or hydroxypropylmethylcellulose phthalate. Dyes or pigments may be added to the tablets or tablet coatings.

Orally administrable pharmaceutical compositions also include hard capsules consisting of gelatin, and also soft, sealed capsules consisting of gelatin and a plasticiser, such as glycerol or sorbitol. The hard capsules may contain the active ingredient in the form of granules, for example in admixture with fillers, such as corn starch, binders, and/or glidants, such as talc or magnesium stearate, and if need be stabilisers. In soft capsules, the active ingredient is preferably dissolved or suspended in suitable liquid excipients, such as fatty oils, paraffin oil or liquid polyethylene glycols or fatty acid esters of ethylene or propylene glycol, to which stabilisers and detergents, for example of the polyoxyethylene sorbitan fatty acid ester type, may also be added.

The formulations suitable for parenteral administration are primarily aqueous solutions of an active ingredient in water-soluble form, e.g. a water-soluble salt, or aqueous injectable suspensions containing viscosity-increasing agents, e.g. sodium carboxymethyl cellulose, sorbitol and/or dextran, and where appropriate stabilisers. Th active ingredient, if need be

together with excipients, can also be in the form of a lyophilisate and can be made into a solution before parenteral administration by the ad dition of suitable solvents.

Solutions such as those used, for example, for parenteral administration can also be employed as infusion solutions.

The invention similarly relates to a process or a method for the treatment of one of the above-mentioned pathological conditions, especially a disease which responds to an enhancement of microtubule polymerisation, especially a corresponding neoplastic disease. A compound of formula IA can be administered as such or in the form of pharmaceutical compositions, prophylactically or therapeutically, preferably in an amount effective against the said diseases, to a warm-blooded animal, for example a human, requiring such treatment, the compounds especially being used in the form of pharmaceutical compositions. In the case of an individual having a bodyweight of about 70 kg the dose administered is from approximately 0.1 mg to approximately 1 g, preferably from approximately 0.5 mg to approximately 200 mg, of a compound of the present invention. Administration is preferably effected e.g. every 1 to 4 weeks.

The present invention also relates in particular to the use of a compound of formula IA, or a pharmaceutically acceptable salt thereof, especially a compound of formula IA named as a preferred compound, or a pharmaceutically acceptable salt thereof, as such or in the form of a pharmaceutical formulation containing at least one pharmaceutically employable carrier, for the therapeutical and also prophylactic treatment of one or more of the above diseases.

The present invention also relates in particular to the use of a compound of formula IA, or a pharmaceutically acceptable salt thereof, especially a compound of formula IA named as a preferred compound, or a pharmaceutically acceptable salt thereof, for the preparation of a pharmaceutical formulation for the therapeutical and also prophylactic treatment of one or more of the above diseases.

The following examples illustrate the invention, but are not intended to restrict their scope in any way.

General: ^1H and ^{13}C NMR spectra were measured in $CDCl_3$, respectively. Positive ion mass spectra, using the fast ion bombardment (FIB) technique, were obtained on a VG ZAB-VSE double focusing, high-resolution mass spectrometer equipped with either a cesium or sodium ion gun. Optical rotations were measured in a one-decimeter (1.3 mL) cell using an Autopol III automatic polarimeter. TLC was performed on glass sheets precoated with silica gel (Merck, Kieselgel 60, F_{254}, Art. 5715). Column chromatographic separations were performed on silica gel (Merck, Kieselgel 60, 230-400 mesh, Art. 9385) under pressure. THF was dried and distilled over sodium ketyl. All antibody reactions were degassed by passing a slow stream of Ar gas into the reaction mixture and carried out in Ar atmosphere. Temperatures are measured in degrees celsius. Unless otherwise indicated, the reactions take place at room temperature.

Abbreviations used:

aqu.	aqueous
BuLi	butyl lithium
CC	column chromatography
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DMF	N,N-dimethylformamide
DIBAL-H	diisobutyl aluminum hydride
DMAP	dimethylaminopyridine
DMSO	dimethyl sulphoxide
EA	ethyl acetate
equiv	equivalent(s)
ESI-MS	Electro-Spray Ionisation Mass Spectroscopy
EtOH	ethanol
EtOAc	acetic acid ethyl ester
HPLC	high pressure liquid chromatography
HRMS	high resolution mass spectrometry
Hünig's base	ethyl diisopropylamine
IC50	concentration leading to 50 % inhibition
LDA	lithium diisopropylamide

Me	methyl
NaHMDS	sodium hexamethyl disilazide
NMR	nuclear magnetic resonance
Ph	phenyl
PBS	phosphate buffered saline
Pr	propyl
PTLC	preparative thin layer chromatography
rt	room temperature
tert	tertiary
TBAF	tetrabutyl ammonium fluoride
TBS or TBDMS	*tert*-butyl-dimethylsilyl
TFA	trifluoroacetic acid
TFAA	trifluoroacetic acid anhydride
THF	tetrahydrofuran
TLC	thin layer chromatography
TMS	tetramethylsilane

Starting Materials - Synthesis of racemic aldols for the Examples 1 to 5.

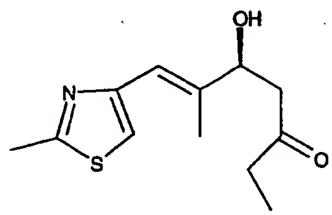
General method: Ketone (1.1 equiv; 2.2 equiv for compounds (±)-**11** of Example 5 and Example 4) is added to a solution of LDA (0.5 M, 1.2 equiv; 2,4 equiv for compounds (±)-**11** of Example 5 and Example 4), freshly prepared from BuLi and i-Pr $_2$NH in THF) at –78 °C. The solution is stirred for 2 h at the same temperature, and then cooled to –100 °C. The corresponding aldehyde (1.0 equiv) in THF is added. The mixture is stirred at – 100 – -80 °C for 0.5 to 1 h, and then quenched with a saturated solution of NH $_4$Cl and allowed to warm to rt. The mixture is diluted with water and extracted with EtOAc. The combined organic layers are washed with brine and dried over $MgSO_4$. Solvents are removed under vacuum and the residue is purified over silica gel (hexane-EtOAc) to afford the pure racemic aldol product.

Compound 12 of Example 5: ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.08 (s, 1H), 6.59 (s, 1H), 4.85 (s, 2H), 4.76 (s, 2H), 4.61 (m, 1H), 3.42 (s, 3H), 3.12 (br s, 1H), 2.72 (m, 2H), 2.20 (s, 3H), 2.04 (s, 3H). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 210.3, 168.0, 154.0, 141.5, 119.3, 117.4, 96.8, 73.2, 66.6, 56.0, 49.0, 31.1, 14.8 ppm.

Compound 13 of Example 5: ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.15 (s, 1H), 6.58 (s, 1H), 5.57 (d, J= 47.0 Hz, 2H), 4.59 (m, 1H), 3.43 (d, J = 2.9 Hz, 1H), 2.70 (m, 2H), 2.19 (s, 3H), 2.01 (s, 3H). ^{13}C NMR (150.9 MHz, $CDCl_3$): δ 209.0, 163.3, 153.4, 141.2, 118.0, 117.7, 81.1, 80.0, 72.5, 48.6, 30.8, 14.8 ppm.

Resolution of Thiazole Aldols (Examples 1 -5)

Example 1:

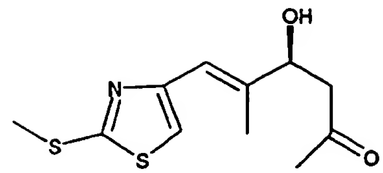


1

Antibody 84G3 (65 mg, 0.000434 mmol) is added to a sterilized solution of 6.43 g (26.9 mmol) of the racemic thiazole aldol (±)-1 in degassed CH_3CN (10 – 20 mL/g aldol) and a degassed buffer (PBS, pH 7.4, 200 mL/g aldol) in a plastic bottle. The mixture is incubated at 37 °C for 5 days. At more than 98% consumption of the *ent*-enantiomer as judged by HPLC analysis, the mixture is filtered using Amicon to recover the antibody and the filtrate is passed through a reverse phase column (C-18) to elute first water and then the organic compounds using methanol as eluants. Solvents are removed under vacuum and the residue is purified by CC (silica gel, hexanes – EtOAc (3:1)) to afford the optically pure aldol compounds **1** (99% ee) and the corresponding aldehyde **1a**. Data for **1**: $[\alpha]_D$ –33.1 (c = 1.28, $CHCl_3$); ^{1}H NMR (400 MHz): δ 6.89 (s, 1H), 6.55 (s, 1H), 4.58 (d, J = 8.6 Hz, 1H), 3.57 (br s, 1H), 2.70 (dd, J = 16.7, 9.4 Hz, 1H), 2.66 (s, 3H), 2.64 (dd, J = 16.7, 3.0 Hz, 1H), 2.47 (q, J = 7.3 Hz, 2H), 2.00 (s, 3H), 1.04 (t, J = 7.3 Hz, 3H); MS: 240 (MH^+), 262 (MNa^+). HPLC (see Ex. 5): Solvent system A, R_t of **1**, 16.20 min and *ent*-1, 18.12 min.

In analogy to the procedure described in Example 1, compounds **2** to **4** are resolved.

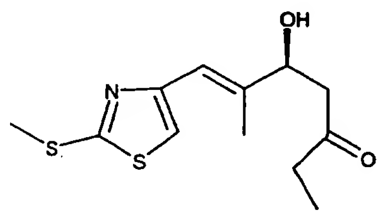
Example 2:



2

3.3 g, 12.8 mmol (±)-2 is resolved by 84G3 (20 mg, 0.000133 mmol) in 7 days to afford 2 with 96% ee; purified by CC (silica gel, hexanes – EtOAc (4:1)). $[\alpha]_D$ –35.2 (c = 2.05, $CHCl_3$). ^{1}H NMR (400 MHz): δ 6.93 (s, 1H), 6.50 (s, 1H), 4.58 (m, 1H), 3.14 (d, J = 3.0 Hz, 1H), 2.70 (d, J = 6.1 Hz, 2H), 2.67 (s, 3H), 2.20 (s, 3H), 2.06 (d, J = 1.2 Hz, 3H); MS: 258 (MH^+), 280 (MNa^+).

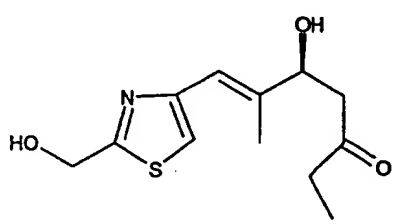
Example 3:



3

8.4 g (30.8 mmol) of (±)-3 is resolved by 84G3 (20 mg, 0.000133 mmol) in 10 days to afford 3 with 99% ee; purified by CC (silica gel, hexanes – EtOAc (4:1)). $[\alpha]_D$ –35.6 (c = 0.92, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.94 (s, 1H), 6.57 (s, 1H), 4.60 (t, J = 6.2 Hz, 1H), 3.32 (br s, 1H), 2.68 (s, 3H), 2.68 (m, 2H), 2.48 (q, J = 7.3 Hz, 2H), 2.07 (s, 3H), 1.07 (t, J = 7.3 Hz, 3H); MS: 272 (MH^+), 294 (MNa^+).

Example 4:



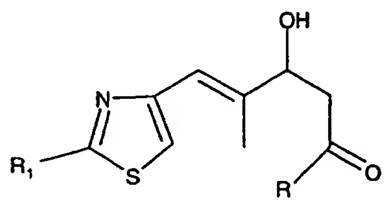
4

8.80 g (34.5 mmol) of (±)-4 is resolved by 84G3 (250 mg, 0.00167 mmol) in 5 days to afford 4 in >99% ee; purified by CC (silica gel, hexanes – EtOAc (1:2)). $[\alpha]_D$

−23.4 (c = 1.08, $CHCl_3$). ^1H NMR (500 MHz): δ 6.94(s, 1H), 6.44 (s, 1H), 5.21 (br s, 1H), 4.76 (s, 2H), 4.50 (d, J = 9.3 Hz, 1H), 3.32 (br, 1H), 2.64 (dd, J = 16.3, 6.8 Hz, 1H), 2.51 (dd, J = 16.3, 3.02 Hz, 1H), 2.42 (q, J = 7.3 Hz, 2H), 1.86 (s, 3H), 0.96 (t, J = 7.3 Hz, 3H); MS: 278 (MNa^+). HPLC (see Ex. 5): Solvent system B, R_t of **4**, 17.10 min and *ent*-**4** 18.42 min.

Example 5:

In analogy to the procedure described in Example 1, compounds of formula (±)-II are resolved by antibody 84G3, 85H6 and 93F3 catalyzed retro-aldol reactions.



((±)-II)

Table 1

compounds of formula (±)-II	84G3 ee [%]	85H6 ee [%]	93F3 ee [%]
5: R = R_1 = Me	98 (50)	94 (50)	98 (50)
6: R = Pr, R_1 = Me	99 (50)	99 (50)	99 (50)
7: R = Bu, R_1 = Me	99 (50)	99 (50)	99 (50)
8: R = Pen, R_1 = Me	97 (52)	97 (55)	97 (53)
9: R = But-1-ene, R_1 = Me	98 (52)	98 (50)	99 (50)
10: R = CH_2F, R_1 = Me	96 (54)	99 (55)	98 (52)
11: R = Me, R_1 = CH_2OH	99 (50)	99 (50)	99 (50)
12: R = Me, R_1 = CH_2OMOM	> 99 (50)	99 (50)	> 99 (51)
13: R = Me, R_1 = CH_2F	> 99 (50)	NT[a]	NT[a]
14: R = Me, R_1 = OMe	>95[b](51)	>95[b](52)	>95[b](52)

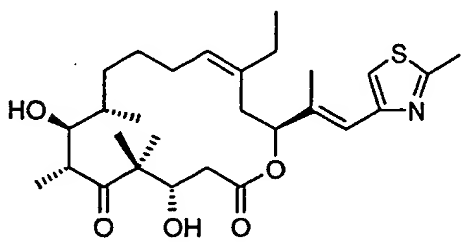
Numbers in parentheses represent the percent conversion.

a: not tested

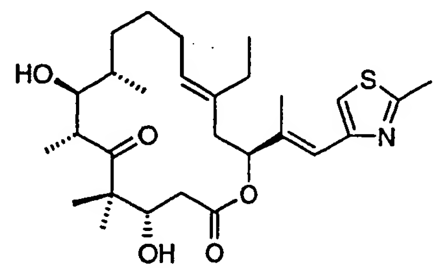
b: Peaks of the two enantiomers on HPLC trace not base-line separable.

HPLC conditions: For compounds of Examples 2 and 3 and compounds 5 to 10 and 14 of Example 5, see Sinha et al, Org. Lett. 1999, 1, 1623 and supporting information; λ_{max} = 254 nm; reverse phase ODR column, Daicel Chemical Industries.
Solvent systems: (A) acetonitrile-water (3:17) and 0.1% TFA, (B) acetonitrile-water (1:4) and 0.1% TFA, (C) acetonitrile-water (3:7) and 0.1% TFA, at a flow rate of 0.4 mL/min.
Compound **11**: Solvent system B, R_t of **11**, 12.56 min and *ent*-**11** 14.38 min. Compound **12**: Solvent system C R_t of **12**, 17.10 min and *ent*-**12** 18.42 min. Compound **13**: Solvent system B, R_t of **13**, 44.27 min and *ent*-**13** 48.72 min.

Example 6:



A



B

TFAA (4 equiv.) is added dropwise to a solution of the 2.8:1 mixture (70 mg, 0.096 mmol) of stage 6.5 in dry CH_2Cl_2 (0.1 M solution) at 0 °C and the mixture is stirred at the same temperature for 4 h and then concentrated under vacuum. The residue is dissolved in EtOAc, washed with brine and dried over $MgSO_4$. Solvents are evaporated and the resulting residue is purified by preparative TLC (PTLC) (silica gel, hexane – EtOAc 2:1) to afford the compounds of the above formula (60 % A, 21 % B)
Physical data of A: $[\alpha]_D$ –69.5° (c = 0.23, $CHCl_3$); ^1H NMR (600 MHz): δ 6.95 (s, 1H), 6.59 (s, 1H), 5.33 (d, J = 10.5 Hz, 1H), 5.20 (d, J = 7.0 Hz, 1H), 4.15 (d, J = 10.9 Hz, 1H), 3.77 (s, 1H), 3.39 (d, J = 4.9 Hz, 1H), 3.11 (qd, J = 7.0, 2.6 Hz, 1H), 2.90 (br s, 1H), 2.84 (dd, J = 14.5, 11.0 Hz, 1H), 2.69 (s, 3H), 2.47 (dd, J = 14.9, 11.0 Hz, 1H), 2.38 (dd, J = 15.4, 2.6 Hz, 1H), 2.21 (m, 1H), 2.08 (s, 3H), 2.03 (m, 3H), 1.93 (m, 1H), 1.75 (m, 1H), 1.73 (s, 3H), 1.59

(m, 1H), 1.39-1.24 (m, 3H), 1.33 (s, 3H), 1.17 (d, J = 6.6 Hz, 3H), 1.10 (s, 3H), 1.00 (d, J = 7.4 Hz, 3H), 0.99 (t, J = 7.4 Hz, 3H); HRMS: ($C_{28}H_{43}NO_5SNa$ = 528.2754) found 528.2735 (MNa^+).

Physical data of B: $[\alpha]_D$ –22.0° (c = 0.10, $CHCl_3$); ^1H NMR (600 MHz): δ 6.96 (s, 1H), 6.56 (s, 1H), 5.46 (d, J = 11.0 Hz, 1H), 5.25 (t, J = 7.0 Hz, 1H), 4.01 (d, J = 10.6 Hz, 1H), 3.76 (m, 1H), 3.21 (quintet, J = 6.6 Hz, 1H), 3.10 (d, J = 2.6 Hz, 1H), 2.70 (s, 3H), 2.54 (dd, J = 15.4, 11.0 Hz, 1H), 2.45 (m, 2H), 2.36 (dd, J = 14.9, 11.0 Hz, 1H), 2.19 (m, 1H), 2.13 (m, 1H), 1.96 (m, 1H), 1.89 (m, 1H), 1.66 (m, 1H), 1.60 (s, 3H), 1.57 (m, 1H), 1.49 (m, 1H), 1.29 (s, 3H), 1.28-1.20 (m, 2H), 1.18 (d, J = 6.5 Hz, 3H), 1.06 (s, 3H), 0.96 (d, J = 7.0 Hz, 3H), 0.94 (t, J = 7.4 Hz, 3H); HRMS: ($C_{28}H_{44}NO_5S$ = 506.2935) found 506.2926 (MH^+).

Stage 6.1

TBSCl (1.5 equiv) is added to a solution of the compound of Example 1 (3.0 g, 12.6 mmol) and imidazole (3.0 equiv) in DMF (2M solution). The reaction mixture is stirred at rt for 24 h and worked up with ether and water. The organic layer is separated and the water phase is extracted with ether. The combined organic layer is washed with brine, dried over $MgSO_4$. Solvents are evaporated and the residue is purified by CC (silica gel, hexanes – EtOAc (10:1)) to afford the pure silyl ether; $[\alpha]_D$ –44.4 (c = 0.98, $CHCl_3$); ^1H NMR (400 MHz): δ 6.89 (s, 1H), 6.50 (s, 1H), 4.63 (dd, J = 9.1, 3.2 Hz, 1H), 2.77 (dd, J = 14.4, 9.4 Hz, 1H), 2.67 (s, 3H), 2.45 (m, 2H), 2.37 (dd, J = 14.4, 3.5 Hz, 1H), 2.00 (d, J = 1.2 Hz, 3H), 1.01 (t, J = 7.0 Hz, 3H), 0.83 (s, 9H), 0.00 (s, 3H), -0.02 (s, 3H); MS: 354 (MH^+).

Stage 6.2

BuLi (1.1 equiv) is added to a heterogeneous mixture of $MePPh_3I$ (1.2 equiv) in dry THF (0.5 M solution) at 0 °C. After the mixture is stirred for 0.5 h at rt, a solution of the compound of stage 6.1 (570 mg, 1.61 mmol) in THF (2 M solution) is added. The reaction mixture is stirred for an additional 0.5 h, and then quenched with a saturated solution of NH_4Cl and extracted with ether. The combined organic layer is washed with brine and dried over $MgSO_4$. Solvents are removed under vacuum and the resultant residue is purified by CC (silica gel, hexanes – EtOAc (20:1)) to afford the pure methylenated product. $[\alpha]_D$ +4.6 (c = 1.00, $CHCl_3$); ^1H NMR (500 MHz): δ 6.90 (s, 1H), 6.45 (s, 1H), 4.75 (m, 2H), 4.23 (dd, J = 7.7, 5.2 Hz, 1H), 2.69 (s, 3H), 2.28 (dd, J = 13.2, 7.7 Hz,

1H), 2.22 (dd, J = 13.2, 5.5 Hz, 1H), 2.05 (q, J = 7.3 Hz, 2H), 1.99 (s, 3H), 1.01 (t, J = 7.3 Hz, 3H), 0.86 (s, 9H), 0.03 (s, 3H), –0.02 (s, 3H); MS: 352 (MH^+).

Stage 6.3

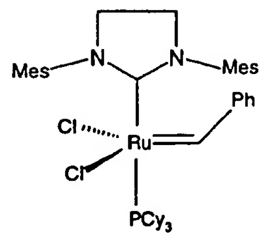
TBAF (1.2 equiv) is added to a solution of the 292 mg (0.83 mmol) of the TBS ether of stage 6.2 in dry THF (0.3 M solution) at 0 °C. After stirred for 1 h at this temperature, the reaction mixture is diluted with water and extracted with EtOAc. The combined organic layer is washed with brine, dried over $MgSO_4$ and solvents are removed. The residue is purified by CC (silica gel, hexanes – EtOAc (4:1)) to afford the pure deprotected product. $[\alpha]_D$ –24.0 (c = 0.73, $CHCl_3$); ^1H NMR (400 MHz): δ 6.89 (s, 1H), 6.55 (s, 1H), 4.84 (d, J = 1.8 Hz, 1H), 4.83 (s, 1H), 4.24 (dd, J = 8.8, 4.1 Hz, 1H), 2.66 (s, 3H), 2.48 (br s, 1H), 2.36 (dd, J = 13.8, 4.1 Hz, 1H), 2.26 (dd, J = 14.1, 9.1 Hz, 1H), 2.05 (q, J = 7.3 Hz, 2H), 2.00 (d, J = 0.9 Hz, 3H), 1.02 (t, J = 7.3 Hz, 3H); ^{13}C NMR (100.6 MHz): δ 164.4, 152.7, 147.8, 141.6, 118.7, 115.3, 111.2, 74.7, 42.9, 28.3, 19.0, 14.2, 12.1; MS: 238 (MH^+), 260 MNa^+).

Stage 6.4

EDC (2.0 equiv) and DMAP (0.1 equiv) are added to a solution of the acid of formula VIII (1.2 equiv) and 43 mg (0.18 mmol) of the thiazole alcohol of stage 6.3 (36 – 41, 1.0 equiv) in dry CH_2Cl_2 (0.2 M solution) at 0 °C. After the reaction mixture is stirred for 16 h at 0 °C to rt, the solvent is evaporated under vacuum and the residue is purified by CC (silica gel, hexanes – EtOAc (10:1)) to afford the corresponding pure ester. $[\alpha]_D$ –43.0 (c = 1.43, $CHCl_3$); ^1H NMR (400 MHz): δ 6.92 (s, 1H), 6.48 (s, 1H), 5.77 (m, 1H), 5.38 (dd, J = 7.9, 6.2 Hz, 1H), 4.97 (dd, J = 17.3, 1.5 Hz, 1H), 4.91 (br d, J = 10.2 Hz, 1H), 4.76 (s, 2H), 4.31 (dd, J = 5.9, 4.1 Hz, 1H), 3.71 (dd, J = 6.7, 2.0 Hz, 1H), 3.14 (quintet, J = 6.7 Hz, 1H), 2.67 (s, 3H), 2.51 (dd, J = 17.3, 3.8 Hz, 1H), 2.46 (dd, J = 14.1, 7.9 Hz, 1H), 2.37 (dd, J = 13.8, 5.9 Hz, 1H), 2.24 (dd, J = 17.3, 5.9 Hz, 1H), 2.06 (d, J = 1.2 Hz, 3H), 2.01 (m, 4H), 1.46 - 1.04 (m, 5H), 1.21 (s, 3H), 1.01 (d, J = 6.4 Hz, 3H), 1.01 (s, 3H), 1.00 (t, J = 7.4 Hz, 3H), 0.87 (d, J = 7.0 Hz, 3H), 0.87 (s, 9H), 0.85 (s, 9H), 0.08 (s, 3H), 0.02 (s, 3H), 0.01 (s, 6H); MS: 784 (MNa^+).

Stage 6.5

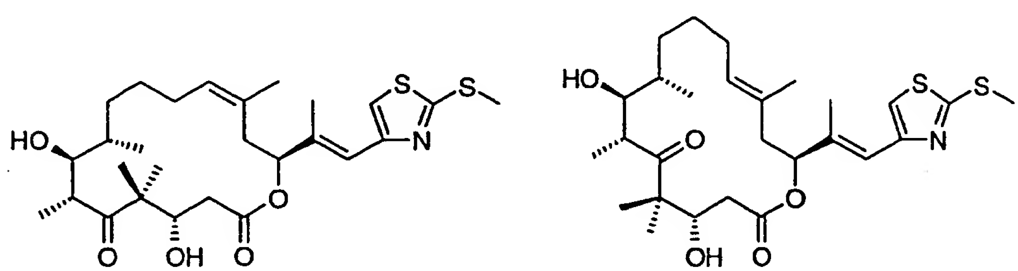
0.2 equiv. of Grubbs' catalyst of formula VI



(VI)

is added to a solution of the diene of stage 6.4 (116 mg, 0.15 mmol) in dry CH_2Cl_2 (0.002 M solution) and the solution is stirred at reflux for 20 - 96 h. After the reaction is completed, as judged by ^{1}H NMR analysis, solvents are evaporated and the residue is purified by CC (silica gel, hexanes – EtOAc (12:1)) to afford a mixture of the metathesized products which is taken to the next step without separation.

Example 7:



A B

The 1.5:1 mixture (77 mg, 0.10 mmol) of stage 7.5 is deprotected according to the method described in the final stage of Example 6 to give pure compounds A (50%) and B (34%); PTLC conditions: silica gel, hexanes – EtOAc (2:1).

Physical data of A: $[\alpha]_D$ −80.6° (c = 0.50, $CHCl_3$); ^{1}H NMR (500 MHz): δ 6.98 (s, 1H), 6.52 (s, 1H), 5.39 (d, J = 10.3 Hz, 1H), 5.21 (d, J = 8.5 Hz, 1H), 4.11 (m, 1H), 3.77 (br s, 1H), 3.11 (qd, J = 6.6, 3.0 Hz, 1H), 2.95 (d, J = 5.5 Hz, 1H), 2.91 (dd, J = 14.7, 11.4, 1H), 2.86 (br s, 1H), 2.69 (s, 3H), 2.49 (dd, J = 15.4, 11.0 Hz, 1H), 2.40 (dd, J = 15.4, 3.0 Hz, 1H), 2.21-2.16 (m, 1H), 2.13 (d, J = 1.5 Hz, 3H), 1.95 (br s, 1H), 1.92 (br s, 1H), 1.78-1.74 (m, 1H), 1.72 (s, 3H), 1.62-1.55 (m, 1H), 1.39-1.24 (m, 3H), 1.32 (s, 3H), 1.17 (d, J = 7.0 Hz, 3H),

1.10 (s, 3H), 1.00 (d, J = 7.0 Hz, 3H); HRMS: ($C_{27}H_{42}NO_5S_2$ = 524.2499) found 524.2522 (MH^+).

Physical data of B: $[\alpha]_D$ –33.3° (c = 0.12, $CHCl_3$); ^{1}H NMR (500 MHz): δ 6.98 (s, 1H), 6.49 (s, 1H), 5.50 (d, J = 9.6 Hz, 1H), 5.31 (m, 1H), 4.00 (d, J = 10.7 Hz, 1H), 3.76 (m, 1H), 3.20 (quintet, J = 6.3 Hz, 1H), 3.02 (m, 1H), 2.69 (s, 3H), 2.56-2.40 (m, 3H), 2.18 (m, 1H), 2.14 (s, 3H), 1.98-1.84 (m, 2H), 1.66 (m, 1H), 1.64 (s, 3H), 1.48 (m, 1H), 1.33 (s, 3H), 1.24 (m, 3H), 1.17 (d, J= 7.0 Hz, 3H), 1.06 (s, 3H), 0.97 (d, J = 7.0 Hz, 3H); HRMS: ($C_{27}H_{42}NO_5S_2$ = 524.2499) found 524.2515 (MH^+).

Stage 7.1

Protection of the compound of Example 2 (1.58 g, 6.1 mmol) is protected according to the method described in stage 6.1 and affords the corresponding silyl ether; CC conditions: silica gel, hexanes – EtOAc (10:1). $[\alpha]_D$ –47.1 (c = 0.95, $CHCl_3$); ^{1}H NMR (400 MHz): δ 6.92 (s, 1H), 6.45 (s, 1H), 4.62 (dd, J = 9.1, 3.5 Hz, 1H), 2.79 (dd, J = 14.7, 9.1 Hz, 1H), 2.69 (s, 3H), 2.44 (dd, J = 14.7, 3.5 Hz, 1H), 2.17 (s, 3H), 2.05 (d, J = 1.5 Hz, 3H), 0.86 (s, 9H), 0.04 (s, 3H), 0.00 (s, 3H); MS: 372 (MH^+).

Stage 7.2

The compound of stage 7.1 (620 mg, 1.67 mmol) is used according to the method described in stage 6.2 to afford the methylenated reaction product; CC conditions: silica gel, hexanes – EtOAc (22:1); $[\alpha]_D$ -9.7 (c = 0.90, $CHCl_3$); ^{1}H NMR (500 MHz): δ 6.92 (s, 1H), 6.40 (s, 1H), 4.73 (m, 2H), 4.24 (dd, J = 7.3, 5.5 Hz, 1H), 2.69 (s, 3H), 2.29 (dd, J = 13.2, 7.7 Hz, 1H), 2.23 (dd, J = 13.2, 4.8 Hz, 1H), 2.04 (d, J = 1.1 Hz, 3H), 1.75 (s, 3H), 0.88 (s, 9H), 0.04 (s, 3H), –0.01 (s, 3H); MS: 370 (MH^+).

Stage 7.3

According to the method described in stage 6.3, the compound of stage 7.2 (498 mg, 1.35 mmol) is deprotected; CC conditions: silica gel, hexanes – EtOAc (5:1); $[\alpha]_D$ –44.8 (c = 1.23, $CHCl_3$); ^{1}H NMR (400 MHz): δ 6.92 (s, 1H), 6.51 (s, 1H), 4.87 (br s, 1H), 4.82 (d, J = 0.9 Hz, 1H), 4.25 (m, 1H), 2.67 (s, 3H), 2.35 (dd, J = 13.5, 4.1 Hz, 1H), 2.25 (dd, J = 14.1, 9.4 Hz, 1H), 2.06 (d, J = 1.2 Hz, 3H), 1.78 (s, 3H); MS: 256 (MH^+).

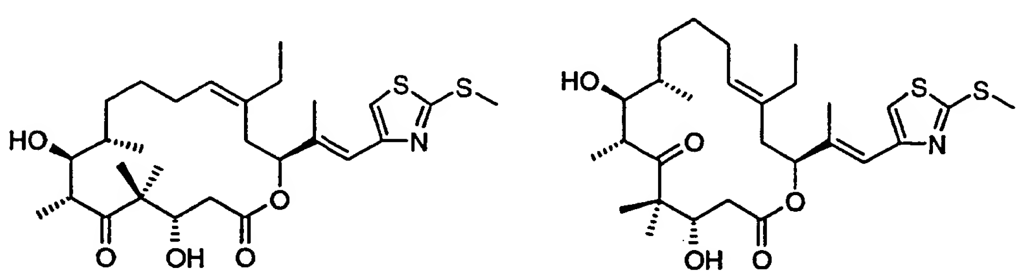
Stage 7.4

According to the method described in stage 6.4, the compound of stage 7.3 (46 mg, 0.18 mmol) is used to afford the corresponding product which is purified by CC (silica gel, hexanes – EtOAc (12:1)); $[\alpha]_D$ –44.4 (c = 1.62, $CHCl_3$); ^{1}H NMR (400 MHz): δ 6.94 (s, 1H), 6.42 (s, 1H), 5.78 (m, 1H), 5.39 (dd, J = 7.6, 4.6 Hz, 1H), 4.97 (dq, J = 17.0, 1.5 Hz, 1H), 4.91 (br d, J = 10.0 Hz, 1H), 4.76 (br s, 1H), 4.74 (br s, 1H), 4.31 (dd, J = 5.9, 3.8 Hz, 1H), 3.71 (dd, J = 6.7, 2.0 Hz, 1H), 3.14 (quintet, J = 6.8 Hz, 1H), 2.67 (s, 3H), 2.53 (dd, J = 17.0, 3.8 Hz, 1H), 2.44 (dd, J = 13.8, 7.9 Hz, 1H), 2.35 (dd, J = 13.8, 5.9 Hz, 1H), 2.25 (dd, J = 17.3, 5.9 Hz, 1H), 2.10 (d, J = 1.2 Hz, 3H), 2.01 (m, 2H), 1.74 (s, 3H), 1.47 – 1.26 (m, 3H), 1.22 (s, 3H), 1.19 – 1.06 (m, 2H), 1.02 (d, J = 5.9 Hz, 3H), 1.01 (s, 3H), 0.87 (d, J = 6.8 Hz, 3H), 0.87 (s, 9H), 0.86 (s, 9H), 0.08 (s, 3H), 0.03 (s, 3H), 0.013 (s, 3H), 0.01 (s, 3H); MS: 802 (MNa^+).

Stage 7.5

According to the method described in stage 6.5, the diene of stage 7.4 (104 mg, 0.13 mmol) is metathesized to afford a mixture of the metathesized products which is taken to the next step without separation; CC conditions: silica gel, hexanes – EtOAc (14:1).

Example 8:



A B

The 2.4:1 mixture (60 mg, 0.078 mmol) of stage 8.5 is deprotected according to the method described in the final stage of Example 6 to yield pure compounds A (59%) and B (24%); PTLC conditions: silica gel, hexanes – EtOAc (3:1).

Physical data of A: $[\alpha]_D$ –64.9° (c = 1.46, $CHCl_3$); ^{1}H NMR (500 MHz): δ 6.98 (s, 1H), 6.51 (s, 1H), 5.34 (d, J = 10.3 Hz, 1H), 5.20 (dd, J =10.7, 3.0 Hz, 1H), 4.10 (m, 1H), 3.78 (m, 1H), 3.11 (qd, J = 7.0, 3.3 Hz, 1H), 2.86 (m, 2H), 2.79 (br s, 1H), 2.69 (s, 3H), 2.48 (dd, J = 15.4,

11.0 Hz, 1H), 2.42 (dd, J = 15.4, 3.0 Hz, 1H), 2.21 (m, 1H), 2.14 (s, 3H), 2.04 (m, 3H), 1.93 (m, 1H), 1.74 (m, 1H), 1.38-1.25 (m, 3H), 1.33 (s, 3H), 1.18 (d, J = 6.6 Hz, 3H), 1.11 (s, 3H), 1.00 (d, J = 7.0 Hz, 3H), 0.99 (t, J = 7.4 Hz, 3H); HRMS: ($C_{28}H_{44}NO_5S_2$ = 538.2655) found 538.2646 (MH^+).

Physical data of B: $[\alpha]_D$ –8.6° (c = 0.40, $CHCl_3$); ^1H NMR (600 MHz): δ 6.98 (s, 1H), 6.49 (s, 1H), 5.46 (d, J = 10.5 Hz, 1H), 5.26 (t, J = 7.0 Hz, 1H), 3.99 (d, J = 10.5 Hz, 1H), 3.75 (m, 1H), 3.20 (quintet, J = 6.6 Hz, 1H), 3.04 (d, J = 2.6 Hz, 1H), 2.70 (s, 3H), 2.53 (dd, J = 14.9, 7.5 Hz, 1H), 2.46 (dd, J = 15.4, 2.3 Hz, 1H), 2.42 (br s, 1H), 2.37 (dd, J = 14.8, 10.9 Hz, 1H), 2.21 (m, 1H), 2.14 (d, J = 0.8 Hz, 3H), 2.12 (m, 2H), 1.95 (dd, J = 14.0, 7.4 Hz, 1H), 1.89 (m, 1H), 1.65 (m, 1H), 1.52-1.45 (m, 1H), 1.29 (s, 3H), 1.27-1.21 (m, 3H), 1.18 (d, J = 7.0 Hz, 3H), 1.06 (s, 3H), 0.96 (d, J = 7.0 Hz, 3H), 0.95 (t, J = 7.2 Hz, 3H); HRMS: ($C_{28}H_{44}NO_5S_2$ = 538.2655) found 538.2651 (MH^+).

Stage 8.1

Protection of the compound of Example 3 (2.36 g, 8.7 mmol) is protected according to the method described in stage 6.1 and affords the corresponding silyl ether; CC conditions: silica gel, hexanes – EtOAc (12:1). $[\alpha]_D$ –54.0 (c = 1.97, $CHCl_3$); ^1H NMR (500 MHz): δ 6.86 (s, 1H), 6.39 (s, 1H), 4.58 (dd, J = 9.1, 3.2 Hz, 1H), 2.73 (dd, J = 14.5, 9.2 Hz, 1H), 2.61 (s, 3H), 2.40 (m, 2H), 2.33 (dd, J = 14.5, 3.3 Hz, 1H), 2.01 (s, 3H), 0.96 (t, J = 7.3 Hz, 3H), 0.79 (s, 9H), -0.04 (s, 3H), -0.06 (s, 3H); MS: 408 (MNa^+).

Stage 8.2

The compound of stage 8.1 (250 mg, 0.65 mmol) is used according to the method described in stage 6.2 to afford the methylenated reaction product; CC conditions: silica gel, hexanes – EtOAc (20:1). $[\alpha]_D$ +0.1 (c = 1.55, $CHCl_3$); ^1H NMR (400 MHz): δ 6.91 (s, 1H), 6.39 (s, 1H), 4.75 (m, 2H), 4.21 (dd, J = 7.3, 5.6 Hz, 1H), 2.69 (s, 3H), 2.26 (m, 2H), 2.05 (q, J = 7.6 Hz, 2H), 2.04 (d, J = 1.1 Hz, 3H), 1.01 (t, J = 7.3 Hz, 3H), 0.87 (s, 9H), 0.02 (s, 3H), –0.02 (s, 3H); MS: 384 (MH^+).

Stage 8.3

According to the method described in stage 6.3, the compound of stage 8.2 (498 mg, 1.35 mmol) is deprotected; CC conditions: silica gel, hexanes – EtOAc (5:1). $[\alpha]_D$ –30.5 (c = 1.13, $CHCl_3$); ^1H NMR (600 MHz): δ 6.92 (s, 1H), 6.50 (s, 1H), 4.88 (m, 1H), 4.85 (br s, 1H), 4.23 (dd, J = 9.7, 4.0 Hz, 1H), 2.67 (s, 3H), 2.38 (dd, J = 14.0, 3.9 Hz, 1H), 2.24 (dd, J

= 14.0, 9.2 Hz, 1H), 2.10 (q, J = 7.5 Hz, 2H), 2.06 (d, J = 1.3 Hz, 3H), 1.03 (t, J = 7.5 Hz, 3H); MS: 270 (MH^+).

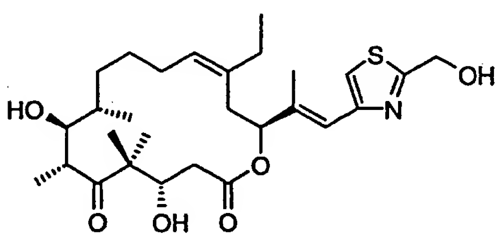
Stage 8.4

According to the method described in stage 6.4, the compound of stage 8.3 (81 mg, 0.3 mmol) is used to afford the corresponding product which is purified by CC (silica gel, hexanes – EtOAc (12:1)); $[\alpha]_D$ – 39.2 (c = 1.10, $CHCl_3$); ^1H NMR (400 MHz): δ 6.93 (s, 1H), 6.41 (s, 1H), 5.76 (m, 1H), 5.37 (dd, J = 7.9, 6.2 Hz, 1H), 4.96 (dq, J = 17.3, 1.5 Hz, 1H), 4.90 (br d, J = 10.2 Hz, 1H), 4.76 (s, 2H), 4.31 (dd, J = 5.9, 4.1 Hz, 1H), 3.70 (dd, J = 6.7, 2.0 Hz, 1H), 3.13 (quintet, J = 7.0 Hz, 1H), 2.67 (s, 3H), 2.50 (dd, J = 17.3, 3.8 Hz, 1H), 2.47 (dd, J = 14.1, 7.9 Hz, 1H), 2.37 (dd, J = 14.1, 5.9 Hz, 1H), 2.24 (dd, J = 17.3, 5.6 Hz, 1H), 2.09 (s, 3H), 2.07 – 1.96 (m, 4H), 1.48 – 1.26 (m, 3H), 1.21 (s, 3H), 1.20 – 1.04 (m, 2H), 1.02 (d, J = 6.4 Hz, 3H), 1.01 (s, 3H), 1.00 (t, J = 7.4 Hz, 3H), 0.88 (d, J = 6.8 Hz, 3H), 0.87 (s, 9H), 0.85 (s, 9H), 0.08 (s, 3H), 0.02 (s, 3H), 0.008 (s, 3H), 0.006 (s, 3H); 828 (MCl^-).

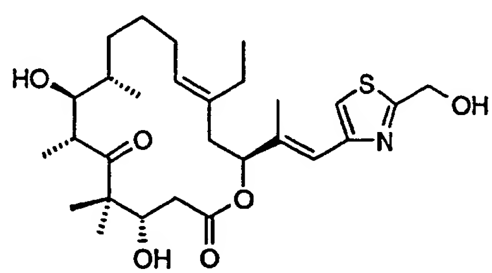
Stage 8.5

According to the method described in stage 6.5, the diene of stage 8.4 (110 mg, 0.14 mmol) is metathesized to afford a mixture of the metathesized products which is taken to the next step without separation; CC conditions: silica gel, hexanes – EtOAc (14:1).

Example 9:



A



B

The 2.3:1 mixture (70 mg, 0.08 mmol) of stage 9.6 is deprotected according to the method described in the final stage of Example 6 to yield pure compounds A (63%) and B (27%); PTLC conditions: silica gel, hexanes – EtOAc (1:2).

Physical data of A: $[\alpha]_D$ –46.1° (c = 0.30, $CHCl_3$); ^{1}H NMR (600 MHz): δ 7.10 (s, 1H), 6.60 (s, 1H), 5.35 (d, J = 10.9 Hz, 1H), 5.20 (d, J = 7.4 Hz, 1H), 4.91 (s, 2H), 4.13 (d, J = 10.9 Hz, 1H), 3.77 (t, J = 3.3 Hz, 1H), 3.28 (br m, 1H), 3.11 (qd, J = 6.8, 3.0 Hz, 1H), 2.85 (dd, J = 14.5, 11.2 Hz, 1H), 2.62 (s, 3H), 2.48 (dd, J = 15.2, 11.2 Hz, 1H), 2.39 (dd, J = 15.3, 2.7 Hz, 1H), 2.20 (m, 1H), 2.09 (s, 3H), 2.07-2.00 (m, 3H), 1.94 (m, 1H), 1.75 (m, 1H), 1.58 (m, 1H), 1.35 (s, 3H), 1.30-1.22 (m, 3H), 1.17 (d, J = 6.8 Hz, 3H), 1.09 (s, 3H), 1.00 (d, J = 7.2 Hz, 3H), 0.99 (t, J = 7.3 Hz, 3H); HRMS: ($C_{28}H_{44}NO_6S$ = 522.2884) found 522.2889 (MH^+).
Physical data of B: $[\alpha]_D$ –18.1° (c = 0.16, $CHCl_3$); ^{1}H NMR (600 MHz): δ 7.11 (s, 1H), 6.58 (s, 1H), 5.45 (d, J = 10.2 Hz, 1H), 5.24 (t, J = 6.6 Hz, 1H), 4.93 (s, 2H), 4.05 (d, J = 10.4 Hz, 1H), 3.77 (s, 1H), 3.75 (d, J = 4.8 Hz, 1H), 3.20 (quintet, J = 6.9 Hz, 1H), 3.05 (br s, 1H), 2.60 (br s, 1H), 2.54 (dd, J = 15.0, 10.6 Hz, 1H), 2.46 (m, 2H), 2.37 (dd, J = 14.4, 10.5 Hz, 1H), 2.25-2.18 (m, 1H), 2.10 (s, 3H), 2.10-2.04 (m, 1H), 1.97 (m, 1H), 1.90 (m, 1H), 1.50 (m, 1H), 1.28 (s, 3H), 1.28-1.20 (m, 4H), 1.18 (d, J = 6.7 Hz, 3H), 1.05 (s, 3H), 0.98 (d, J = 7.0 Hz, 3H), 0.95 (t, J = 6.5 Hz, 3H); HRMS: ($C_{28}H_{44}NO_6S$ = 522.2884) found 522.2888 (MH^+).

Stage 9.1

Protection of the compound of Example 4 (3.2 g, 12.5 mmol) is protected according to the method described in stage 6.1 and affords the corresponding silyl ether; CC conditions: silica gel, hexanes – EtOAc (20:1). $[\alpha]_D$ –32.1 (c = 1.21, $CHCl_3$); ^{1}H NMR (400 MHz): δ 7.00 (s, 1H), 6.50 (s, 1H), 4.93 (s, 2H), 4.63 (dd, J = 9.4, 3.2 Hz, 1H), 2.77 (dd, J = 14.4, 9.4 Hz, 1H), 2.46 (m, 2H), 2.37 (dd, J = 14.3, 3.2 Hz, 1H), 2.00 (s, 3H), 1.01 (t, J = 7.0 Hz, 3H), 0.93 (s, 9H), 0.83 (s, 9H), 0.11 (s, 6H), 0.01 (s, 3H), –0.02 (s, 3H); MS: 484 (MH^+), 506 (MNa^+).

Stage 9.2

The compound of stage 9.1 (547 mg, 1.13 mmol) is used according to the method described in stage 6.2 to afford the methylenated reaction product; CC conditions: silica gel, hexanes – EtOAc (40:1). $[\alpha]_D$ +1.5 (c = 2.53, $CHCl_3$); ^{1}H NMR (400 MHz): δ 7.01 (s, 1H), 6.44 (s, 1H), 4.95 (s, 2H), 4.75 (m, 2H), 4.22 (dd, J = 7.6, 5.6 Hz, 1H), 2.29 (dd, J = 13.6, 7.6 Hz, 1H), 2.24 (dd, J = 13.6, 5.6 Hz, 1H), 2.05 (q, J = 7.3 Hz, 2H), 1.99 (s, 3H), 1.01 (t, J = 7.3 Hz, 3H), 0.95 (s, 9H), 0.87 (s, 9H), 0.12 (s, 6H), 0.02 (s, 3H), –0.03 (s, 3H); MS: 482 (MH^+), 504 (MNa^+).

Stage 9.3

According to the method described in stage 6.3 but using 2.4 equiv. of TBAF instead of 1.2 equiv., the compound of stage 9.2 (180 mg, 0.37 mmol) is deprotected; CC conditions: silica gel, hexanes – EtOAc (1:2). $[\alpha]_D$ –23.3 (c = 1.33, $CHCl_3$); ^{1}H NMR (400 MHz): δ 6.99 (s, 1H), 6.49 (s, 1H), 4.82 (m, 5H), 4.22 (dd, J = 8.5, 4.4 Hz, 1H), 3.08 (br s, 1H), 2.34 (dd, J = 14.1, 4.4 Hz, 1H), 2.24 (dd, J = 14.1, 8.8 Hz, 1H), 2.04 (q, J = 7.3 Hz, 2H), 1.93 (s, 3H), 1.01 (t, J = 7.3 Hz, 3H); MS: 254 (MH^+), 276 (Mna^+).

Stage 9.4

In a manner as described under stage 11.4 the compound of stage 9.3 (80 mg, 0.32 mmol) is allowed to react with TBSCl (60 mg, 0.38 mmol) and *i*-Pr_2NEt (0.11 mL, 0.64 mmol) in dry CH_2Cl_2 (3 mL) at 0 °C to afford the protected derivative which is purified by CC (silica gel, hexanes – EtOAc, (3:1)); $[\alpha]_D$ –23.2 (c = 0.50, $CHCl_3$); ^{1}H NMR (500 MHz): δ 7.03 (s, 1H), 6.57 (s, 1H), 4.94 (s, 2H), 4.89 (d, J = 1.5 Hz, 1H), 4.86 (s, 1H), 4.25 (dd, J = 9.2, 3.7 Hz, 1H), 2.39 (dd, J = 14.0, 3.7 Hz, 1H), 2.27 (dd, J = 14.0, 9.2 Hz, 1H), 2.09 (q, J = 7.4 Hz, 2H), 2.04 (d, J = 1.1 Hz, 3H), 1.05 (t, J = 7.5 Hz, 3H), 0.94 (s, 9H), 0.12 (s, 6H); ^{13}CNMR (125.75 MHz): δ 171.9, 153.1, 147.9, 141.5, 118.8, 115.6, 111.4, 74.7, 63.2, 43.1, 28.4, 25.7, 18.2, 14.4, 12.2, –5.5; MS: 390 (MNa^+).

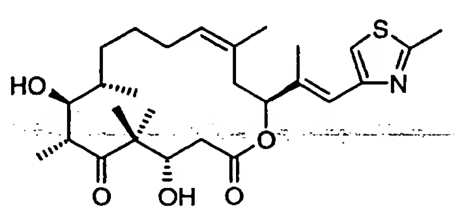
Stage 9.5

According to the method described in stage 6.4, the compound of stage 9.4 (46 mg, 0.13 mmol) is used to afford the corresponding product which is purified by CC (silica gel, hexanes – EtOAc (15:1). $[\alpha]_D$ –35.4 (c = 1.00, $CHCl_3$); ^{1}H NMR (400 MHz): δ 7.03 (s, 1H), 6.48 (s, 1H), 5.78 (m, 1H), 5.39 (dd, J = 7.6, 6.2 Hz, 1H), 4.97 (dq, J = 17.0, 1.4 Hz, 1H), 4.94 (s, 2H), 4.92 (br d, J = 10.2 Hz, 1H), 4.77 (s, 2H), 4.31 (dd, J = 5.6, 3.8 Hz, 1H), 3.71 (dd, J = 7.0, 2.4 Hz, 1H), 3.14 (quintet, J = 6.7 Hz, 1H), 2.51 (dd, J = 17.0, 3.8 Hz, 1H), 2.47 (dd, J = 15.0, 8.2 Hz, 1H), 2.36 (dd, J = 15.0, 5.8 Hz, 1H), 2.25 (dd, J = 17.0, 5.9 Hz, 1H), 2.05 (s, 3H), 2.00 (m, 4H), 1.48 – 1.25 (m, 5H), 1.22 (s, 3H), 1.02 (d, J = 6.8 Hz, 3H), 1.01 (s, 3H), 1.00 (t, J = 7.3 Hz, 3H), 0.94 (s, 9H), 0.88 (s, 9H), 0.87 (d, J = 6.7 Hz, 3H), 0.86 (s, 9H), 0.12 (s, 6H), 0.09 (s, 3H), 0.02 (s, 3H), 0.01 (s, 6H); MS: 892 (MH^+).

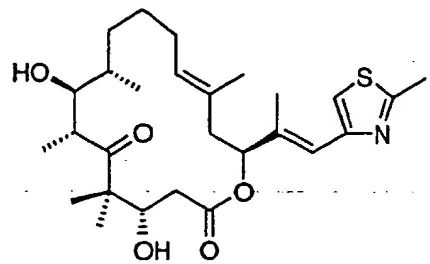
Stage 9.6

According to the method described in stage 6.5, the diene of stage 9.5 (90 mg, 0.1 mmol) is metathesized to afford a mixture of the metathesized products which is taken to the next step without separation; CC conditions: silica gel, hexanes – EtOAc (20:1).

Example 10:



A



B

HF-pyridine (0.5 mL) is added to a solution of the 1.2:1 mixture (70 mg, 0.097 mmol) of stage 10.5 in dry THF (0.05 M solution) at rt. The solution is stirred at the same temperature for 6 h. The completion of the reaction is judged by TLC. The reaction mixture is slowly poured into a cold aqueous solution of $NaHCO_3$ and extracted with EtOAc. The organic layer is washed with brine and dried over $MgSO_4$. Solvents are evaporated under reduced pressure. The residue is purified by PTLC (silica gel, hexanes – EtOAC (2:1)) to yield the pure epothilones A (54%) and B (45%).

Physical data of A: $[\alpha]_D$ –71.6 (c = 0.50, $CHCl_3$); ^1H NMR (600 MHz): δ 6.95 (s, 1H), 6.60 (s, 1H), 5.38 (d, J = 11.1 Hz, 1H), 5.20 (d, J = 7.0 Hz, 1H), 4.17 (d, J = 10.9 Hz, 1H), 3.76 (br s, 1H), 3.47 (m, 1H), 3.11 (m, 1H), 2.96 (br s, 1H), 2.89 (dd, J = 14.3, 11.5 Hz, 1H), 2.68 (s, 3H), 2.48 (dd, J = 15.0, 11.4 Hz, 1H), 2.37 (dd, J = 15.0, 2.3 Hz, 1H), 2.17 (m, 1H), 2.08 (s, 3H), 1.97 – 1.86 (m, 4H), 1.74 (m, 1H), 1.72 (s, 3H), 1.57 (m, 1H), 1.35 – 1.20 (m, 3H), 1.33 (s, 3H), 1.17 (d, J = 6.9 Hz, 3H), 1.09 (s, 3H), 0.99 (d, J = 7.1 Hz, 3H); HRMS: ($C_{27}H_{42}NO_5S$ = 492.2784; $C_{27}H_{41}NO_5SNa$ = 514.2603;) found 492.2760 (MH^+), 514.2589 (MNa^+).
Physical data of B: $[\alpha]_D$ –30.2 (c = 0.5, $CHCl_3$); ^1H NMR (600 MHz): δ 6.96 (s, 1H), 6.56 (s, 1H), 5.49 (dd, J = 10.8, 1.1 Hz, 1H), 5.29 (m, 1H), 4.01 (dt, J = 10.7, 2.5 Hz, 1H), 3.75 (br s, 1H), 3.19 (quintet, J = 6.1 Hz, 1H), 3.13 (d, J = 3.2 Hz, 1H), 2.69 (s, 3H), 2.53 (dd, J = 14.9, 10.7 Hz, 1H), 2.45 (dd, J = 14.7, 2.4 Hz, 1H), 2.44 (t, J = 11.0 Hz, 1H), 2.32 (m, 2H), 2.20 (m, 1H), 2.08 (d, J = 0.9 Hz, 3H), 1.87 (m, 1H), 1.82 (br s, 1H), 1.63 (m, 1H), 1.62 (s, 3H),

1.55 (m, 1H), 1.47 (m, 1H), 1.28 (s, 3H), 1.22 (m, 1H), 1.17 (d, J = 6.8 Hz, 3H), 1.10 (m, 1H), 1.05 (s, 3H), 0.96 (d, J = 7.0 Hz, 3H); HRMS: ($C_{27}H_{42}NO_5S$ = 492.2784; $C_{27}H_{41}NO_5SNa$ = 514.2603); found 492.2798 (MH^+), 514.2640 (MNa^+).

Stage 10.1

Protection of the compound 5 of Example 5 is protected according to the method described in stage 6.1 and affords the corresponding silyl ether.

Stage 10.2

The compound of stage 10.1 (500 mg, 1.48 mmol) is used according to the method described in stage 6.2 to afford the methylenated reaction product; CC conditions: silica gel, hexanes – EtOAc (20:1). $[\alpha]_D$ +1.0 (c = 0.50, $CHCl_3$); ^1H NMR (500 MHz): δ 6.90 (s, 1H), 6.45 (s, 1H), 4.75 (m, 1H), 4.70 (m, 1H), 4.25 (dd, J = 7.7, 5.9 Hz, 1H), 2.68 (s, 3H), 2.29 (dd, J = 13.6, 7.7 Hz, 1H), 2.22 (dd, J = 13.6, 5.5 Hz, 1H), 1.99 (s, 3H), 1.74 (s, 3H), 0.87 (s, 9H), 0.04 (s, 3H), –0.02 (s, 3H); MS: 338 (MH^+).

Stage 10.3

According to the method described in stage 6.3, the compound of stage 10.2 (400 mg, 1.19 mmol) is deprotected; CC conditions: silica gel, hexanes – EtOAc (4:1). $[\alpha]_D$ –28.8 (c = 1.87, $CHCl_3$); ^1H NMR (500 MHz): δ 6.88 (s, 1H), 6.54 (s, 1H), 4.83 (s, 1H), 4.79 (s, 1H), 4.25 (dd, J = 8.8, 4.4 Hz, 1H), 2.65 (s, 3H), 2.49 (br s, 1H), 2.32 (dd, J = 13.6, 4.4 Hz, 1H), 2.26 (dd, J = 14.0, 8.8 Hz, 1H), 1.99 (s, 3H), 1.75 (s, 3H); MS: 224 (MH^+).

Stage 10.4

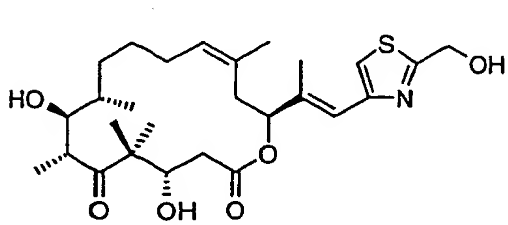
According to the method described in stage 6.4, the compound of stage 10.3 (35 mg, 0.16 mmol) is used to afford the corresponding product which is purified by CC (silica gel, hexanes – EtOAc, (10:1)); $[\alpha]_D$ –34.7 (c = 1.95, $CHCl_3$); ^1H NMR (400 MHz): δ 6.92 (s, 1H), 6.48 (s, 1H), 5.76 (m, 1H), 5.40 (dd, J = 7.6, 5.9 Hz, 1H), 4.98 (dd, J = 17.3, 2.1 Hz, 1H), 4.90 (br d, J = 10.3 Hz, 1H), 4.75 (s, 1H), 4.73 (s, 1H), 4.31 (dd, J = 5.6, 3.8 Hz, 1H), 3.71 (dd, J = 7.0, 2.4 Hz, 1H), 3.13 (quintet, J = 6.7 Hz, 1H), 2.67 (s, 3H), 2.50 (dd, J = 17.0, 3.8 Hz, 1H), 2.45 (dd, J = 13.8, 7.9 Hz, 1H), 2.34 (dd, J = 13.8, 5.6 Hz, 1H), 2.25 (dd, J = 17.0, 5.6 Hz, 1H), 2.05 (d, J = 1.2 Hz, 3H), 1.99 (m, 2H), 1.73 (s, 3H), 1.46-1.05 (m, 5H), 1.21 (s, 3H), 1.01 (d, J = 5.9 Hz, 3H), 1.01 (s, 3H), 0.87 (s, 9H), 0.87 (d, J = 6.5 Hz, 3H), 0.85 (s, 9H), 0.08 (s, 3H), 0.02 (s, 3H), 0.01 (s, 6H); ^{13}C NMR (100.6 MHz): δ 217.6, 171.1,

164.4, 152.5, 140.9, 138.8, 137.2, 121.0, 116.3, 114.3, 113.7, 77.61, 77.56, 74.0, 53.3, 45.2, 41.7, 40.3, 38.7, 34.3, 34.3, 31.5, 30.4, 27.0, 26.1, 26.0, 23.0, 22.6, 22.4, 20.3, 19.2, 18.4, 18.1, 17.6, 15.4, 14.4, 14.1, –3.7, –3.8, –4.4, –4.8; MS: 770 (MNa^+).

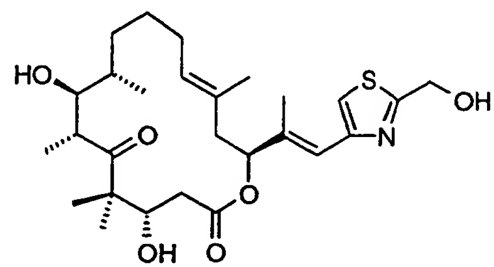
Stage 10.5

According to the method described in stage 6.5, the diene of stage 10.4 (94 mg, 0.13 mmol) is metathesized to afford a mixture of the metathesized products which is taken to the next step without separation; CC conditions: silica gel, hexanes – EtOAc (12:1).

Example 11:



A



B

The 1.5:1 mixture (100 mg, 0.12 mmol) of stage 11.6 is deprotected according to the method described in the final stage of Example 10 to yield pure compounds A (55%) and B (36%); PTLC conditions: silica gel, hexanes – EtOAc (1:2).

Physical data of A: $[\alpha]_D$ –70.0° (c = 0.29, $CHCl_3$); ^1H NMR (600 MHz): δ 7.10 (s, 1H), 6.60 (s, 1H), 5.39 (d, J = 10.7 Hz, 1H), 5.21 (d, J = 9.0 Hz, 1H), 4.91 (s, 2H), 4.16 (d, J = 9.8 Hz, 1H), 3.75 (m, 1H), 3.37 (br s, 1H), 3.11 (qd, J = 6.9, 2.7 Hz, 1H), 2.93 (br s, 1H), 2.89 (dd, J = 14.6, 11.3 Hz, 1H), 2.62 (s, 1H), 2.48 (dd, J = 15.3, 11.4 Hz, 1H), 2.37 (dd, J = 15.1, 2.6 Hz, 1H), 2.16 (d, J = 3.1 Hz, 3H), 1.94 (m, 2H), 1.77-1.73 (m, 1H), 1.72 (s, 3H), 1.58 (m, 1H), 1.34 (s, 3H), 1.24 (br s, 4H), 1.17 (d, J = 6.9 Hz, 3H), 1.09 (s, 3H), 0.97 (d, J = Hz, 3H); HRMS: ($C_{27}H_{41}NO_6SNa$ = 530.2547) found 530.2544 (MNa^+). Physical data of B: $[\alpha]_D$ –36.6° (c = 0.24, $CHCl_3$); ^1H NMR (600 MHz): δ 7.11 (s, 1H), 6.59 (s, 1H), 5.49 (d, J = 10.5 Hz, 1H), 5.29 (t, J = 6.8 Hz, 1H), 4.92 (s, 2H), 4.06 (d, J = 10.6 Hz, 1H), 3.78 (br s, 1H), 3.75 (m, 1H), 3.20 (quintet, J = 6.6 Hz, 1H), 3.08 (br s, 1H), 2.62 (s, 1H), 2.54 (dd, J = 15.0, 10.7 Hz, 1H), 2.47 (m, 2H), 2.35 (m, 2H), 2.20 (m, 1H), 2.16 (s, 3H), 1.89 (m, 1H), 1.62 (s, 3H), 1.60-1.44

(m, 2H), 1.28 (s, 3H), 1.24 (br s, 4H), 1.18 (d, J = 6.8 Hz, 3H), 1.05 (s, 3H), 0.97 (d, J = 7.0 Hz, 3H); HRMS: ($C_{27}H_{42}NO_6S$ = 508.2727) found 508.2730 (MH^+).

Stage 11.1

Protection of the compound 11 of Example 5 is protected according to the method described in stage 6.1 and affords the corresponding silyl ether.

Stage 11.2

The compound of stage 11.1 (715 mg, 1.52 mmol) is used according to the method described in stage 6.2 to afford the methylenated reaction product; CC conditions: silica gel, hexanes – EtOAc (40:1). $[\alpha]_D$ –1.3 (c = 1.33, $CHCl_3$); ^1H NMR (400 MHz): δ 7.01 (s, 1H), 6.44 (s, 1H), 4.95 (s, 2H), 4.75 (m, 1H), 4.71 (br s, 1H), 4.24 (dd, J = 7.3, 5.3 Hz, 1H), 2.29 (dd, J = 13.6, 7.3 Hz, 1H), 2.21 (dd, J = 13.6, 5.3 Hz, 1H), 1.98 (s, 3H), 1.74 (s, 3H), 0.94 (s, 9H), 0.86 (s, 9H), 0.12 (s, 6H), 0.03 (s, 3H), –0.02 (s, 3H); ^{13}C NMR (100.6 MHz): δ 171.8, 153.3, 142.4, 142.3, 118.9, 115.1, 113.1, 77.6, 63.2, 45.5, 25.8, 25.7, 22.8, 18.2, 13.7, –4.7, –5.1, –5.5; MS: 468 (MH^+), 490 (MNa^+).

Stage 11.3

According to the method described in stage 6.3 but using 2.4 equiv. of TBAF instead of 1.2 equiv., the compound of stage 11.2 (492 mg, 1.05 mmol) is deprotected; CC conditions: silica gel, hexanes – EtOAc (1:2). $[\alpha]_D$ –35.0 (c = 0.48, $CHCl_3$); ^1H NMR (400 MHz): δ 7.01 (s, 1H), 6.53 (s, 1H), 4.85 (br s, 3H), 4.80 (s, 1H), 4.25 (dd, J = 8.2, 4.1 Hz, 1H), 2.32 (dd, J = 13.8, 4.4 Hz, 1H), 2.26 (dd, J = 13.5, 8.8 Hz, 1H), 1.95 (s, 3H), 1.76 (s, 3H); MS: 240 (MH^+), 262 (MNa^+).

Stage 11.4

TBSCl (140 mg, 0.93 mmol) is added to a solution of the compound of stage 11.3 (180 mg, 0.75 mmol) and *i*-Pr_2NEt (0.26 mL, 1.50 mmol) in dry CH_2Cl_2 (5 mL) at 0 °C and the mixture is stirred at 0 °C to rt for 8h. The reaction mixture is worked up with water and CH_2Cl_2. The combined organic layer is washed with water and dried over $MgSO_4$. Solvents are evaporated and the protected derivative is purified by CC (silica gel, hexanes – EtOAc, 3:1); $[\alpha]_D$ -26.3 (c = 1.54, $CHCl_3$); ^1H NMR (500 MHz): δ 7.03 (s, 1H), 6.57 (s, 1H), 4.94 (s, 2H), 4.88 (s, 1H), 4.83 (s, 1H), 4.27 (dd, J = 9.2, 4.4 Hz, 1H), 2.35 (dd,

J = 14.0, 4.4 Hz, 1H), 2.28 (dd, J = 14.0, 9.2 Hz, 1H), 2.04 (s, 3H), 1.79 (s, 3H), 0.94 (s, 9H), 0.12 (s, 6H); MS: 354 (MH^+).

Stage 11.5

According to the method described in stage 6.4, the compound of stage11.4 (65 mg, 0.18 mmol) is used to afford the corresponding product which is purified by CC (silica gel, hexanes – EtOAc (15:1)); $[\alpha]_D$ –38.2 (c = 1.03, $CHCl_3$); ^1H NMR (500 MHz): δ 7.03 (s, 1H), 6.49 (s, 1H), 5.77 (m, 1H), 5.41 (dd, J = 7.7, 5.9 Hz, 1H), 4.97 (dd, J = 17.3, 1.5 Hz, 1H), 4.93 (s, 2H), 4.92 (br d, J = 10.3 Hz, 1H), 4.76 (s, 1H), 4.74 (s, 1H), 4.30 (dd, J = 5.6, 3.8 Hz, 1H), 3.72 (dd, J = 7.0, 2.2 Hz, 1H), 3.14 (quintet, J = 6.6 Hz, 1H), 2.53 (dd, J = 17.2, 4.0 Hz, 1H), 2.45 (dd, J = 14.0, 8.1 Hz, 1H), 2.36 (dd, J = 14.0, 5.9 Hz, 1H), 2.26 (dd, J = 16.9, 5.5 Hz, 1H), 2.05 (s, 3H), 2.00 (m, 2H), 1.74 (s, 3H), 1.48 – 1.27 (m, 3H), 1.22 (s, 3H), 1.15 – 1.04 (m, 2H), 1.02 (d, J = 6.3 Hz, 3H), 1.01 (s, 3H), 0.94 (s, 9H), 0.88 (s, 9H), 0.87 (d, J = 6.3 Hz, 3H), 0.86 (s, 9H), 0.12 (s, 6H), 0.09 (s, 3H), 0.03 (s, 3H), 0.02 (s, 3H), 0.016 (s, 3H); MS: 878 (MH^+).

Stage 11.6

According to the method described in stage 6.5, the diene of stage 11.5 (125 mg, 0.14 mmol) is metathesized to afford a mixture of the metathesized products which is taken to the next step without separation; CC conditions: silica gel, hexanes – EtOAc (20:1).

Example 12

The efficacy of compounds of formula I as inhibitors of micro tubule depolymerisation and the efficacy against tumour cells was tested in the test procedures as described herein-above.

Table 2

Example	Tubulin Polymerization (%)	KB-31 Cell Growth Inhibition (IC50 [nM])[a]
6A	11	150
6B	33	78.9
7A	39	24.8
7B	78	4.03

8A	54	47.9
8B	64	17.5
10A	2	245
10 B	19	61.6

(a) Drug concentration required for half-maximal inhibition of KB-31 human epidermoid cancer cell growth was assessed after a 72h drug exposure by quantification of cell mass using a protein dye method.

Example 13: Dry capsules

3000 capsules, each of which contain 0.25 g of one of the compounds of the formula IA mentioned in the preceding Examples as active ingredient, are prepared as follows:

Composition

Active ingredient	75.00 g
Lactose	750.00 g
Avicel PH 102 (microcrystalline cellulose)	300.00 g
Polyplasdone XL (polyvinylpyrrolidone)	30.00 g
Magnesium stearate	9.00 g

Preparation process: The active ingredient is passed through a No. 30 hand screen. The active ingredient, lactose, Avicel PH 102 and Polyplasdone XL are blended for 15 minutes in a mixer. The blend is granulated with sufficient water (about 500 mL), dried in an oven at 35°C overnight, and passed through a No. 20 screen.

Magnesium stearate is passed through a No. 20 screen, added to the granulation mixture, and the mixture is blended for 5 minutes in a mixer. The blend is encapsulated in No. 0 hard gelatin capsules each containing an amount of the blend equivalent to 25 mg of the active ingredient.

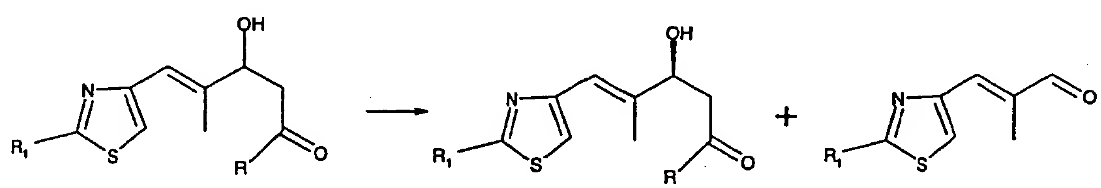
What is claimed is:

1. A process for enantioselectively resolving a racemic mixture of an aldol synthon, the racemic mixture including a first and a second enantiomer of the aldol synthon, said process comprising the following steps:

Step A: Catalyzing a retro-aldol reaction for enantioselectively converting the first enantiomer of the aldol synthon to form an aldehyde product while leaving the second enantiomer of the aldol synthon unmodified, said catalyst employing a catalytic antibody; and then

Step B: Separating the aldehyde product from the unmodified second aldol synthon.

2. A process according to claim 1 wherein the racemic mixture of the aldol synthon of formula (±)-II is resolved to provide an unmodified enantiomer of formula II* and an aldehyde of formula III



(±)-II II* III

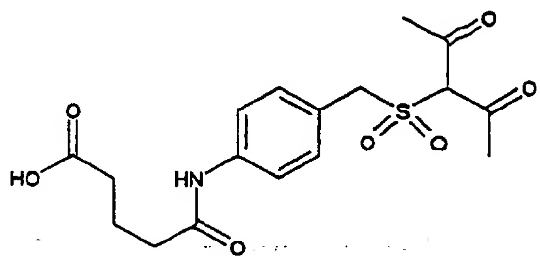
3. A process according to claim 2 wherein
R_1 represents methyl, hydroxymethyl, halomethyl, methylthio or methoxy, and
R represents C_{1-5} alkyl, n-but-1-en-4-yl or halomethyl.

4. A process according to claim 3 wherein
R_1 represents methyl, hydroxymethyl, methylthio or methoxy, and
R represents methyl, ethyl, n-propyl, n-butyl, n-pentyl or n-but-1-en-4-yl.

5. A process according to any one of claims 1 to 4 wherein th catalytic antibody is selected from the group consisting of 84G3, 85H6 and 93F3.

6. A catalytic antibody selected from the group consisting of 84G3, 85H6 and 93F3.

7. Use of a compound of formula IV



(IV).

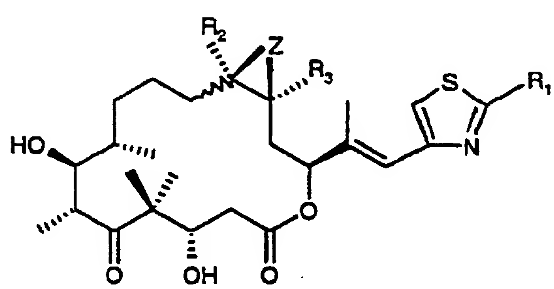
for the preparation of monoclonal antibodies using reactive immunization.

8. A compound of formula II*, wherein

R_1 represents methyl, hydroxymethyl, halomethyl, lower alkoxy lower alkylenoxy methyl, methylthio or methoxy, and

R represents lower alkyl, n-but-1-en-4-yl or halomethyl.

9. A process for the preparation of a compound of formula I,



(I)

in which compound

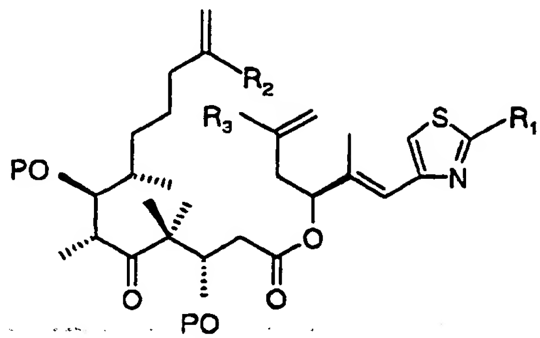
R_1 is methyl, hydroxymethyl, halomethyl, methylthio or methoxy;

R_2 is hydrogen, methyl,

R_3 is hydrogen or lower alkyl, and

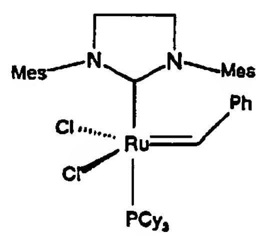
Z is O or a bond;

wherein a compound of formula V,



(V)

in which P is tert-butyl-dimethylsilyl or another suitable protecting group for a hydroxy group, R_1 is methyl, hydroxymethyl which is protected by tert-butyl-dimethylsilyl or another suitable protecting group for a hydroxy group, halomethyl, methylthio or methoxy, and the other radicals have the meaning as given above for formula I, is transformed into a compound of formula I by an olefine methathesis reaction in the presence of the catalyst of formula VI



(VI)

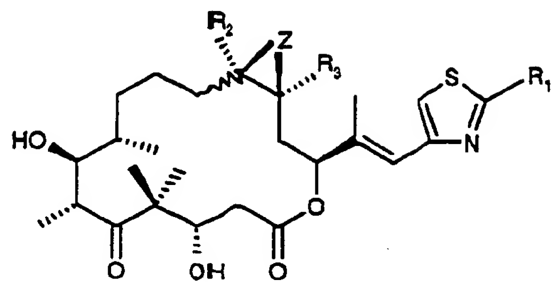
in which formula Mes represents mesityl and Ph phenyl,

followed by the detaching of the protecting group by a suitable reagent,

and in which process directly before or after detaching the protecting groups present a compound of formula I wherein Z represents a bond can optionally be transformed by epoxidation into a compound of formula I wherein Z represents O,

and, after carrying out the above process, if necessary for the preparation of a salt, converting a resulting free compound of the formula I into a salt or, if necessary for preparation of a free compound, converting a resulting salt of a compound of the formula I into the free compound.

10. A compound of formula IA



(IA)

wherein

R_1 is methyl, hydroxymethyl, halomethyl, methylthio or methoxy;

R_2 is hydrogen or methyl,

R_3 is lower alkyl, and

Z is O or a bond;

or a pharmaceutically acceptable salt thereof.

11. A compound according to claim 10 of formula IA wherein

R_1 is methylthio;

R_2 is hydrogen;

R_3 is lower alkyl, and

Z is O or a bond;

or a pharmaceutically acceptable salt thereof.

12. A pharmaceutical composition, comprising a compound of formula IA or a pharmaceutically acceptable salt thereof, provided that at least one salt-forming groups is present, according to claim 10 or 11, and one or more pharmaceutically acceptable carriers.

13. A compound of formula IA according to claim 10 or 11, for use in a process for the diagnostic or therapeutic treatment of humans.

14. Use of a compound of formula IA according to claim 10 or 11 for the treatment of a tumour disease.

15. Use of a compound of formula IA according to claim 10 or 11 for the preparation of a pharmaceutical product for the treatment of a tumour disease.

16. A method for treatment of warm-blooded animals, including humans, in which an anti-tumourally effective dose of a compound of the formula IA according to claim 10 or 11 or of a pharmaceutically acceptable salt of such a compound is administered to a warm-blooded animal suffering from a tumour disease.

17. Use according to claim 15 or a method according to claim 16 wherein the tumour disease is breast cancer or prostate cancer.